SEXUAL SELECTION IN THE SANDFLY *LUTZOMYIA LONGIPALPIS*

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This thesis is dedicated to my parents
whose constant love and support has
given me the courage to get this far.

Abstract

In this thesis I address several fundamental questions in sexual selection and mate choice theory, using the sandfly *Lutzomyia longipalpis*. I show that *L. longipalpis* satisfies the criteria for a lek-breeding species. Males aggregated at sites near hosts which females visited to mate and feed, but a male's access to a resource did not appear to determine his mating success. Females were free to reject unwanted males and male mating success was typically skewed. Field data suggest that the mating system may have evolved because of female preferences for larger leks, coupled with a hotspot-type mechanism: male distribution was correlated with resources availability, while females distribution was correlated with lek size. In the laboratory, females preferentially mated with middle-aged males. Within this age class, mating success was correlated with increased amounts of pheromone and increased investment in wing-fluttering. Across age classes, females appeared to gain a direct fitness payoff from their choice of mate through increased probability of fertilisation, but the presence of other mechanisms was not investigated. By testing simultaneously the predictions of current models using similar age males, I was able to assess their relative importance for the maintenance of female choice. I found weak evidence to support direct benefits: females that chose to mate with successful males survived longer post-oviposition than females with less successful mates. This did not translate into increased total longevity or increased fecundity. I found no evidence in favour of good-genes models: offspring of preferred males did not survive longer than offspring of less preferred males, nor were their daughters more fecund. Male attractiveness was, however, heritable: sons sired by preferred males achieved higher mating success than sons of less preferred fathers. These results suggest that a Fisherian mechanism is in part responsible for the maintenance of female mating preferences in *L. longipalpis*.

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Therésa

XXX

Chapter 1

Introduction

It is over a century since Darwin suggested that sexual selection arises from "the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction" (Darwin 1871, p 256). Although sexual selection is now more usually viewed as a subset of natural selection (Andersson 1994), Darwin made an explicit distinction between the two in order to explain the evolution of exaggerated male traits which appeared to reduce survival. Sexual selection, he said, "depends, not on the struggle for existence, but on a struggle between males for the possession of females; the result is not death to the competitor, but few or no offspring" (Darwin 1859, p 88). Essentially Darwin envisaged two processes driving the evolution of exaggerated male traits: mate competition and mate choice. While the importance of competition between individuals for access to mates is generally accepted, the role of mate choice in sexual selection remains controversial. Why should individuals (usually females) express a preference for particular members of the opposite sex (usually males)?

Female choice

Female choice is usually defined as "any pattern of behaviour shown by [females] that leads to their being more likely to mate with certain [males] than with others" (Halliday 1983). Despite initial scepticism about its existence (reviewed by Pomiankowski 1988, Cronin 1991) female choice for certain males and the traits that they possess has now been reported across a range of species (see Andersson 1994, pp 132-142). Choice may be based on single or multiple traits (Møller and Pomiankowski 1993, Andersson and Iwasa 1996), and in some species a female may use these cues in a hierarchy to select a suitable mate (reviewed by Gibson and Langen 1996: e.g. see Sivinski *et al.* 1984). However, despite the plethora of empirical observations, our theoretical understanding of female choice - of the mechanisms which females use to sample males (Janetos 1980, Gibson and Langen 1996), and in particular of the fitness consequences of female mating preferences - remains a key area of debate (Kirkpatrick and Ryan 1991, Maynard-Smith 1991, Williams 1992).

Evolution of female choice

One of the most contentious issues in sexual selection is how female preferences for particular male traits evolve and are maintained (reviewed in Andersson 1994). In resource-based mating systems, where choosy females gain directly through access to a good quality territory, or to high quality parental care for example, the fitness benefits of choice are obvious. However, female choice is also pronounced in some non-resource based systems, such as leks. In lekking species, males aggregate at sites (or leks) which females visit ostensibly for the purpose of mating. The territories of males contain no resources essential for breeding females and males provide no parental care (Bradbury 1981). Despite the absence of direct benefits, females continue to exhibit a high degree of unanimity in their choice of mates and are frequently more discriminating than females of resource-defending species (Reynolds and Gross 1990). This has been termed the "paradox of the lek" (Taylor and Williams 1982, Williams 1992).

Fisher (1915, 1930, 1958) was the first to suggest how female preferences for particular male traits could be maintained in the absence of direct fitness payoffs. He proposed that if a male trait evolved which was preferred by certain females, then females with a strong mate preference would produce attractive sons with higher mating success. Both trait and preference would thus spread through the population. As an alternative he suggested that a female preference for a male trait might evolve if the trait in some way indicates high viability which is in turn inherited by the offspring of choosy females. Only the first of these theories of "indirect selection" is usually attributed to Fisher (see Andersson 1994, p 27); viability indicator mechanisms or "good-genes" models for the evolution of female choice are usually considered as more recent ideas (Zahavi 1975, Hamilton and Zuk 1982).

When these hypotheses were initially proposed they raised a serious objection. In the absence of direct benefits, it was predicted that, under strong selection, additive genetic variance in the male trait would be rapidly driven to fixation (Maynard Smith 1978, Charlesworth 1987, Taylor and Williams 1982).

Recent models have shown, however, that additive genetic variation is rarely completely diminished (Iwasa *et al.* 1991, Rowe and Houle 1996) and therefore indirect selection is sufficient to promote the evolution of exaggerated males traits and maintain female preferences for them (Kirkpatrick 1987, Pomiankowski and Møller 1995, Rowe and Houle 1996). Moreover, it has been suggested that sexually selected traits may have higher additive genetic variance than non-sexually selected traits in a similar taxa (Pomiankowski and Møller 1995, but see Rowe and Houle 1996).

Empirical support for theories of indirect sexual selection remains equivocal. Because of the necessity to infer past history from the present state using a phylogenetic framework, testing the evolution of a female preference is often troublesome. This is exemplified by the controversy over whether the female preference for males possessing swordtails in the genus *Xiphophorus* pre-dates the origin of the sword itself (Basolo 1990, Meyer *et al.* 1994, Basolo 1995). Furthermore, several of the critical predictions proposed to distinguish between alternative models for the evolution of female choice have similar outcomes and thus would not, in practice, do so (Kirkpatrick and Ryan 1991, Balmford and Read 1991).

Largely because of these problems, the majority of empirical studies have instead focused on testing the predictions for the maintenance of female preferences. A positive genetic correlation between the female preference and the preferred male trait has been demonstrated in several species (three-spined sticklebacks, *Gasterosteus aculeatus*: Bakker 1993; guppy, *Poecilia reticulata*: Houde and Endler 1990, Houde 1994; and the stalk eyed fly, *Cyrtodiopsis dalmanni*: Wilkinson and Reillo 1994). However, such a correlation is a central prediction of both good genes and Fisherian mechanisms (Iwasa *et al.* 1991). Most recent empirical tests of good-genes models have thus investigated correlations between the preferred male trait and male viability (Milinski and Bakker 1990, von Schantz *et al.* 1994, Kotiaho *et al.* 1996), and a few studies report enhanced survival of offspring sired by preferred males (Boake 1986, Simmons 1987, Norris

1993, Petrie 1994, Gilburn *et al.* 1996). Support for a Fisherian mechanism is, however, lacking (Bakker and Pomiankowski 1995). Only one study, to my knowledge, has tested directly, and failed to find, heritability of male mating success (Whittier and Kaneshiro 1995). An important limitation in all these studies testing indirect selection is that they fail to assess the relative importance of these processes by examining all the potential fitness payoffs simultaneously (Andersson 1994). Non-resource based mating systems, such as leks, provide an ideal opportunity to study directly these genetic benefits of female choice, without the potentially confounding effects of resource acquisition.

Mate choice on leks

Male mating success is typically highly skewed on leks (Bradbury *et al.* 1985, Wiley 1991, Höglund and Alatalo 1995), such that a few males obtain a high proportion of available matings while the majority obtain few or no matings. Although leks appear to combine pronounced female choice with an absence of any direct fitness gains to choosy females, several factors complicate the interpretation of the maintenance of female mating preferences in lekking species. For instance, in some species, males appear to disrupt matings to such an extent that some authors have questioned how far lekking females are free to choose their mates at all (Diamond 1981, Trail 1985, McComb and Clutton-Brock 1994). Mate choice copying may also lead to an exaggerated impression of the degree of skew in independent female choice (Clutton-Brock *et al.* 1988, Wade and Pruett-Jones 1990, Gibson and Höglund 1992). In addition, there are growing suggestions that even lekking females may gain direct benefits from their choice of mate in terms of increased probability of fertilisation, or reduced risks of harassment, predation, or risk of infection with a sexually transmitted disease (Wrangham 1980, Borgia and Collis 1990, Clutton-Brock *et al.* 1988, McComb and Clutton-Brock 1994; see also Kirkpatrick and Ryan 1991, Höglund and Alatalo 1995). Clearly all these issues need to be examined in any experimental studies using leks to test the current theories of sexual selection.

Why use a lek-breeding insect for studying sexual selection?

Lekking has been reported in mammals, birds, fish and a diverse array of insects (see Höglund and Alatalo 1995). The majority of insects for which lek formation been described belong to the order Diptera, although there are several lek-breeding species of Lepidoptera and Hymenoptera. Lekking insects are frequently used in answering questions in sexual selection because of several important advantages over other taxa. First, they have short generation times and thus the consequences of selection can be explored over several generations within a relatively short time-frame. Second, they can often be reared and manipulated in the laboratory allowing carefully designed experiments to be performed with comparative ease. Third, these experiments can often require large numbers of individuals. Recent examples of the use of insects in studies of sexual selection include estimating the relative importance of male competitive interactions and female choice in determining male reproductive success (Hawaiian *Drosophila*, *Drosophila grimshawi*, Droney 1992); exploring the co-evolution of exaggerated male traits and female preference for them (stalk-eyed fly, *Cyrtodiopsis dalmanni*: Wilkinson and Reillo 1994); and testing whether choosy females gain indirect fitness benefits through enhanced offspring fitness (Mediterranean fruit fly, *Ceratitis capitata*: Whittier and Kaneshiro 1995; seaweed fly, *Coleopa frigida*: Gilburn *et al.* 1996).

Aims and thesis outline

In this thesis I present a series of experiments using the phlebotomine sandfly *Lutzomyia longipalpis*, which has recently been described as breeding on leks (Jarvis and Rutledge 1992) aimed at addressing fundamental questions in sexual selection and mating system theory. The thesis is organised as follows. In Chapter 2, I briefly review the species biology of *L. longipalpis* and provide baseline data for techniques which will then be referred to throughout the thesis. Chapter 3 examines how well *L. longipalpis* fits the criteria of a lekking species as defined by Bradbury (1981). In particular, I explore whether male competitive interactions or female choice determine a male's reproductive success, and whether the variance in mating success is dependent on the presence of available resources.

Two key results that emerge are that *L. longipalpis* aggregations are leks *sensu strictu*, and that females do exhibit pronounced preferences for particular males, even when they are all of similar age. In Chapter 4, I test the current predictions for the evolution of lekking by manipulating the availability of resources in the field, and examining the consequences for the distribution of males and females. The final three data chapters focus on the cues used in mate choice and the possible fitness consequences of female mating preferences. In Chapter 5 I look at how female choice might be influenced by male age. Females visiting leks are exposed to males of different ages (Kruijt and de Voss 1988, Alatalo *et al.* 1992). Because a male's age may affect his ability to fertilise females, one potential benefit to females of age related choice might be an increased probability of fertilisation (Williams 1992). I explore these two ideas by allowing females to choose males differing in age, and then investigating the consequences in terms of fertilisation success. In Chapter 6 I explore why females discriminate between males even when they are the same age. The experiment is the first thorough test of the relative importance of all potential direct and indirect benefits models of female choice, and looks at the fitness consequences of female choice in terms both of the survival and fecundity of the females themselves, and the survival and reproductive success of her offspring. Finally, because little is known about what cues *L. longipalpis* might use in mate choice, but males are known to emit pheromones which attract both sexes (Ward *et al.* 1988), Chapter 7 is devoted to testing the possible role of pheromonal cues in determining male reproductive success. I control for the potentially confounding effect of age by restricting investigation to the most preferred age group. I conclude in Chapter 8 with a review of the most important findings of the thesis and a discussion of possible avenues for future research.

Chapter 2

Species biology

Summary

1. The chapter begins with a brief review of the current literature on the adult and larval ecology of the species of phlebotomine sandfly used in this thesis, *Lutzomyia longipalpis* (Diptera: Psychodidae). Males form large aggregations at or near hosts in the field and recent laboratory evidence suggests that these aggregations resemble leks (Jarvis and Rutledge 1992). Attraction of both sexes to a site is mediated by pheromones emitted by males, as well as by host odours. Both sexes communicate acoustically by fluttering their wings. Little is known about the larval ecology of this species, although larvae are suspected to be aggregated in distribution.
2. In the second half of the chapter, I describe a set of preliminary experiments aimed at answering four important questions about the natural history, culture and manipulation of my study species. First, I explored how offspring survival varied with different initial egg density. I found no evidence to suggest that survival was density dependent.
3. Second, I tested whether I could mark individuals by examining the effect of fluorescent powders on male behaviour and survival. Marking did not appear to affect male behaviour, but males marked with yellow powder survived less well than unmarked control males.
4. In order to investigate whether sperm depletion occurs, a third experiment involved mating males with ten virgin females in short succession. The first six females mated all had an equal probability of being fertilised by a male.
5. The final experiment looked at whether individual females mated multiply. Females were exposed to aggregations of males, 1hr, 24hr and 72hr after their first mating. A total of 16 of 81 females mated for a second time and the second matings were significantly shorter than first matings. Mated females also appeared to be less responsive: they remained motionless in the cage and did not approach or wing-flutter (fan their wings) at males. Virgin females wing-fluttered at approaching males and were active until they mated.

The study species

What are sandflies?

The phlebotomine sandflies are a well-defined subfamily of the family Psychodidae (Order: Diptera). To date, approximately 600 species of phlebotomines have been described, from six genera. About half of the identified species occur in the genera *Lutzomyia* and *Lutzomyia longipalpis* (Lutz and Neiva) is one of the better-studied species.

Lutzomyia longipalpis

Lutzomyia longipalpis (Figure 2.1) is distributed from northern Argentina to Mexico. As with the majority of sandflies, females are haematophagous, requiring a bloodmeal for the development of their eggs, while adults of both sexes feed on plant nectars for general metabolism. Laboratory evidence suggests that the number of eggs produced varies with the size of the bloodmeal obtained (Ready 1979), and egg development takes approximately three days. On average in the laboratory 62.5% (50/80) of mature eggs are laid and the remainder are retained in the female (Killick-Kendrick *et al.* 1977). These retention figures are much higher than any I have observed (Chapters 5 and 6), but in my experience females also appear to produce less eggs, suggesting that differences in levels of bloodfeeding may account for this pattern. It is likely that egg retention is an artefact induced by the laboratory environment because females with retained eggs are rare in the field (Killick-Kendrick *et al.* 1977). When laid, eggs are white, but within a few hours they darken to brown. Larvae pass through four instars, feeding on decaying organic matter (which can be replaced with dried liver powder in the laboratory), before pupating. Pupae generally stand upright, securing themselves to a surface by means of their larval exuviae, which are retained at the end of the abdomen. Development from egg to adult takes approximately 6-8 weeks at 25^{0}C, 80% humidity (Killick-Kendrick *et al.* 1977). Mortality in the laboratory is high: on average 18-25% of eggs laid survive to emerge as adults (Killick-Kendrick *et al.* 1977). Egg hatch, development from the first to second larval instar, egg retention

and death of engorged females prior to oviposition are the most common causes of mortality in the laboratory (Killick-Kendrick *et al.* 1977).

Adult ecology

While sylvatic in origin, *L. longipalpis* has become well established in the peridomestic environment, where they aggregate on or near hosts that are housed predominantly in chicken sheds (Lainson *et al.* 1990, Quinnell and Dye 1994a, Quinnell and Dye 1994b). The ecology of the adult is relatively well known. In the field, flies are active between 18:00 and 06:00 hr (Quinnell and Dye 1994a). Males arrive at sheds earlier in the evening than females and form relatively stable male-biased aggregations (Kelly and Dye 1997). Attraction of both sexes to a host is mediated by pheromones emitted by males (Ward *et al.* 1988, Elnaiem and Ward 1991, Ward and Morton 1991) as well as by host odours (Elnaiem and Ward 1991, Oshagi et al. 1994, Hamilton and Ramsoondar 1994). The data suggest a degree of site fidelity: males return to the same chicken sheds on consecutive nights (Kelly and Dye 1997), and there are consistent differences between sites in the numbers of flies caught (Quinnell and Dye 1994a, Quinnell and Dye 1994b, Kelly *et al.* 1996, Chapters 3 and 4). Females visit aggregations of males near hosts to obtain a bloodmeal and to mate. By examining the number of folicular relics in the ovaries of females caught in light traps, Dye *et al.* (1987) estimated that the life-expectancy of field-caught females is approximately three gonotrophic cycles. However, at present, there is no equivalent technique for ageing field-caught males.

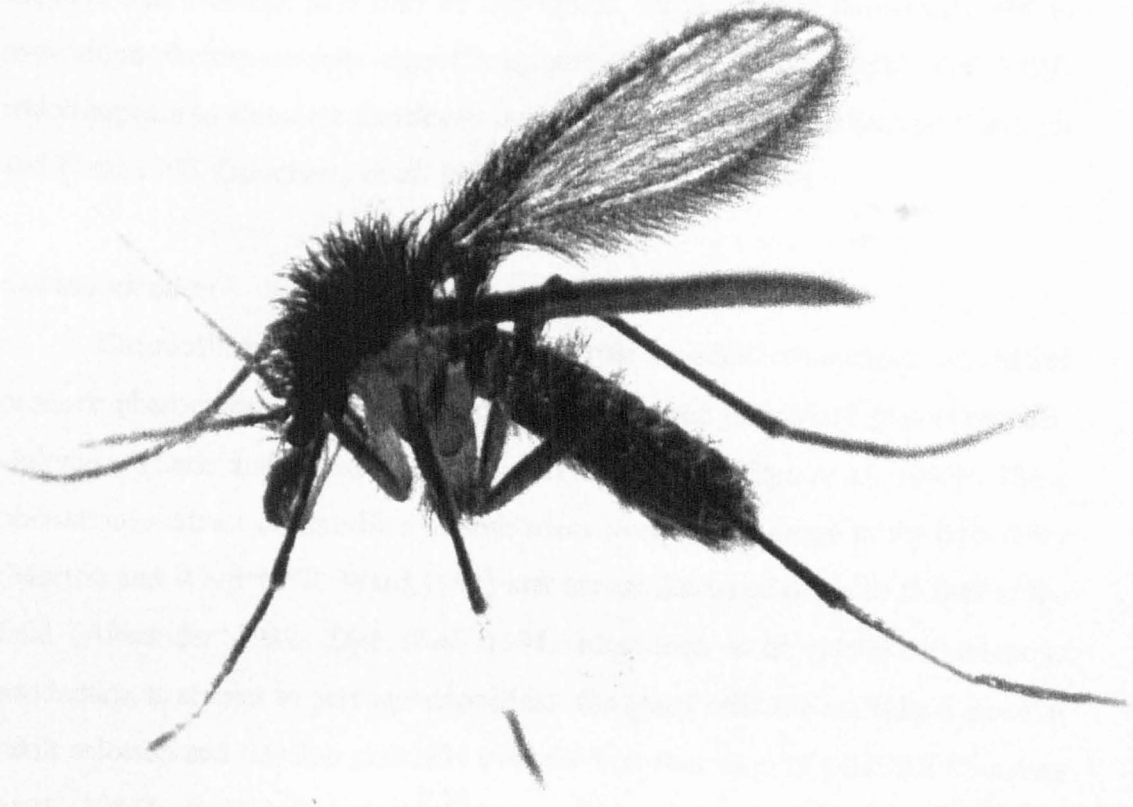


Figure 2.1. Female *Lutzomyia longipalpis* (magnification: x30).

Larval ecology

While the distributions of nocturnal aggregations are well documented, daytime resting sites and particularly oviposition sites are still unknown, and so there is little field data on the larval ecology of *L. longipalpis* (see Killick-Kendrick 1978 for a review). It is believed that the larval stage of the life-cycle lasts approximately 5-6 weeks (Morrisson *et al.* 1993), which is comparable with laboratory estimates (Killick-Kendrick *et al.* 1977). Laboratory evidence also suggests that breeding sites may be aggregated, since cultured females secrete an oviposition pheromone onto eggs (Dougherty *et al.* 1992, Dougherty *et al.* 1994) which appears to stimulate females to oviposit in aggregated distributions (Elnaiem and Ward 1992, Dougherty *et al.* 1993, Dougherty *et al.* 1994).

Communication

Chemical signals play an important role in adult communication. Males produce pheromones that are produced by and stored in pairs of glands on their abdomens (Lane and Ward 1984, Lane *et al.* 1985, Philips *et al.* 1986). These pheromones attract conspecifics of both sexes over a short range in the laboratory (Morton and Ward 1990, Ward 1991) and across distances of up to 0.5km in the field (Alexander 1987, Dye *et al.* 1991, Morrisson *et al.* 1993). Pheromone production is at least in part age-dependent: the gland cells are not fully formed at adult eclosion and develop gradually over the first four days of adult life (Boufana *et al.* 1986). It is believed that pheromones may be involved in speciation (Hamilton *et al.* 1996), but no study has yet attempted to investigate whether they have a role in mate choice. Furthermore, virtually nothing is known about the method of pheromone dispersal, although it is suspected that it is aided by wing-fluttering displays of males, where wing-fluttering is defined as when a male fans his wings either in isolation or at an approaching fly.

In addition to pheromone communication, both sexes produce song by fluttering their wings (Ward *et al.* 1988). The role of song in mate choice and conspecific attraction is even less well studied than the role of pheromones. There is evidence to suggest that sibling species differ in the length of the interval

between each song burst, and that females prefer the song of conspecifics (Ward *et al.* 1988). However, acoustic communication does not appear to be a necessary precursor for copulation as wingless males obtain matings (Ward *et al.* 1988).

Mating system

It has been suggested that the aggregations of *L. longipalpis* males observed in the laboratory and field resemble leks (Jarvis and Rutledge 1992), as defined by Bradbury (1981). Jarvis and Rutledge (1992) investigated the distribution and reproductive success of males and females in a caged laboratory environment. Males preferentially aggregated on hosts (mice) that females visited to feed and mate. Once on a host, males defended small territories, wing-fluttering and performing hopping displays at approaching males. Occasionally, these displays escalated to a fight, where two or more males made contact, tumbling over one another and clashing abdomens. The degree of male aggregation was dependent on the age of the males present: older males were more likely to aggregate than average age or young males. Jarvis and Rutledge's study (1992) is limited - although it concludes that *L. longipalpis* exhibits a lek-like mating system, it fails to demonstrate that all the necessary criteria of a lekking species are satisfied. For example, the role of a host (a resource) in determining male reproductive success is unknown. How far females are free to choose their mates unconstrained by male aggression is also unclear.

As well as *L. longipalpis*, at least one other sandfly species, *Phlebotomus argentipes*, is known to form male-biased aggregations on or near hosts (Lane *et al.* 1989) in the field. In addition, several species have been observed to aggregate on tree buttresses (Memmott 1991, 1992, Memmott and Sutton 1993). To date, however, there is little published data on the dynamics of the mating system of any of these other species.

Sibling species

The interpretation of data on the behaviour and ecology of *L. longipalpis* is made more difficult by the observation that it forms a species complex. Mangabeira

(1969) noted that while geographically isolated females do not appear to be morphologically distinct, males vary in the number of pairs of small pale patches present on their abdomens. Males from certain regions bear a pair on both the 3rd and 4th abdominal tergites, while others bear a pair on just the 4th segment. Analysis of these patches showed that they are sites of pheromone production (Lane *et al.* 1984, Lane *et al.* 1985, Phillips *et. al.* 1986). Subsequent laboratory crosses suggest that species recognition depends not on the morphology of these patches but rather on differences in the chemistry of the pheromones produced (Ward *et al.* 1983, Ward *et al.* 1988, Hamilton *et al.* 1996). Genetic studies (Lanzaro *et al.* 1995) and salivary analysis (Warburg *et al.* 1994) have also aided in identification of at least five geographically isolated sibling species. In addition, auditory communication may have a role in species recognition (Ward *et al.* 1988).

The study population

The population used throughout this thesis originated in the Salvaterra district of Marajó Island, Brazil (48°31'S, 0°46'W), where it is thought that only one member of the species complex is present. All Salvaterra males that have been analysed are of a single pheromone type, Cembrene-B (Hamilton and Pickett unpub. data), and bear a single pair of glands on the 4th tergite. The laboratory colony was initially set up using eggs laid by approximately 100 wild caught females; this stock had been maintained in the laboratory for twenty generations prior to the start of this project. Field studies (Chapters 3 and 4) were carried out in Salvaterra, using wild caught flies.

Culturing techniques

In Brazil, flies were maintained at ambient temperature and humidity and on a natural diurnal cycle (approximately 26-30°C, 70-80% humidity, 12:12hr light:dark cycle). Emerging adults were released into mixed-sex net cages (dimensions: 15x15x15cm; Figure 2.2a). Unless otherwise stated this cage size is used throughout all future experiments. Cages were enclosed in plastic bags containing moistened cottonwool (to maintain humidity), and flies were fed daily with cottonwool swabs soaked in sugar solution.

Groups of 20-30 females were bloodfed 3-5d after emergence on a mouse or hamster. The animal was anaesthetised using the procedure described by Flecknell (1987) and then placed on its back in a net cage (Figure 2.2a) and exposed to feeding females for 30min. Fed females were transferred to glass jars (approximate dimensions: 6cm diameter, 10cm height) covered in netting for oviposition. Moistened folded filter paper was placed in the jars to increase the surface area for egg laying (Elnaiem and Ward 1992). After all females had died, all eggs were removed and transferred in batches of 200-300 to Petri dishes (10cm diameter 1.5cm height), lined with filter paper, and covered with netting to prevent escape of emerging larvae. Eggs were checked daily for the presence of first instar larvae and signs of fungus. When larvae hatched they were provided sparingly with larval food (dried liver powder). If fungal growth appeared, small amounts of sand were sprinkled over the dish to reduce the growth of hyphae (Ward *et al.* 1988, R. Killick-Kendrick pers. comm.). To avoid desiccation, Petri-dishes were maintained with a dish of distilled water in polystyrene-lined cabinets. Using this method, females laid an average (± s.e.) of 42.7 (±1.6) eggs (n = 50 females) and retained an average of 0.7(±0.4) eggs. Development time from female feeding to adult emergence was approximately 5-6 weeks and of all eggs laid by females, 17%± 0.03 % (mean ± s.e.) emerged as adults.

This technique was modified in three ways in the UK. (Note that unless otherwise stated, experiments included in this thesis were undertaken in Brazil.)

(i) All flies were maintained in an incubator at 25-26^{0}C, 80% humidity, and on a 12:12 hr light:dark cycle.

(ii) Bloodfed females were placed into Hilton pots (8cm height, 12cm diameter) lined with plaster of Paris (described in Killick-Kendrick *et al.* 1973, 1977), to further reduce fungal growth which is a particular problem in an incubator (Killick-Kendrick *et al.* 1977).

(iii) Hilton pots were maintained in plastic boxes lined with moist sand to minimise the risk of desiccation (Figure 2.3).

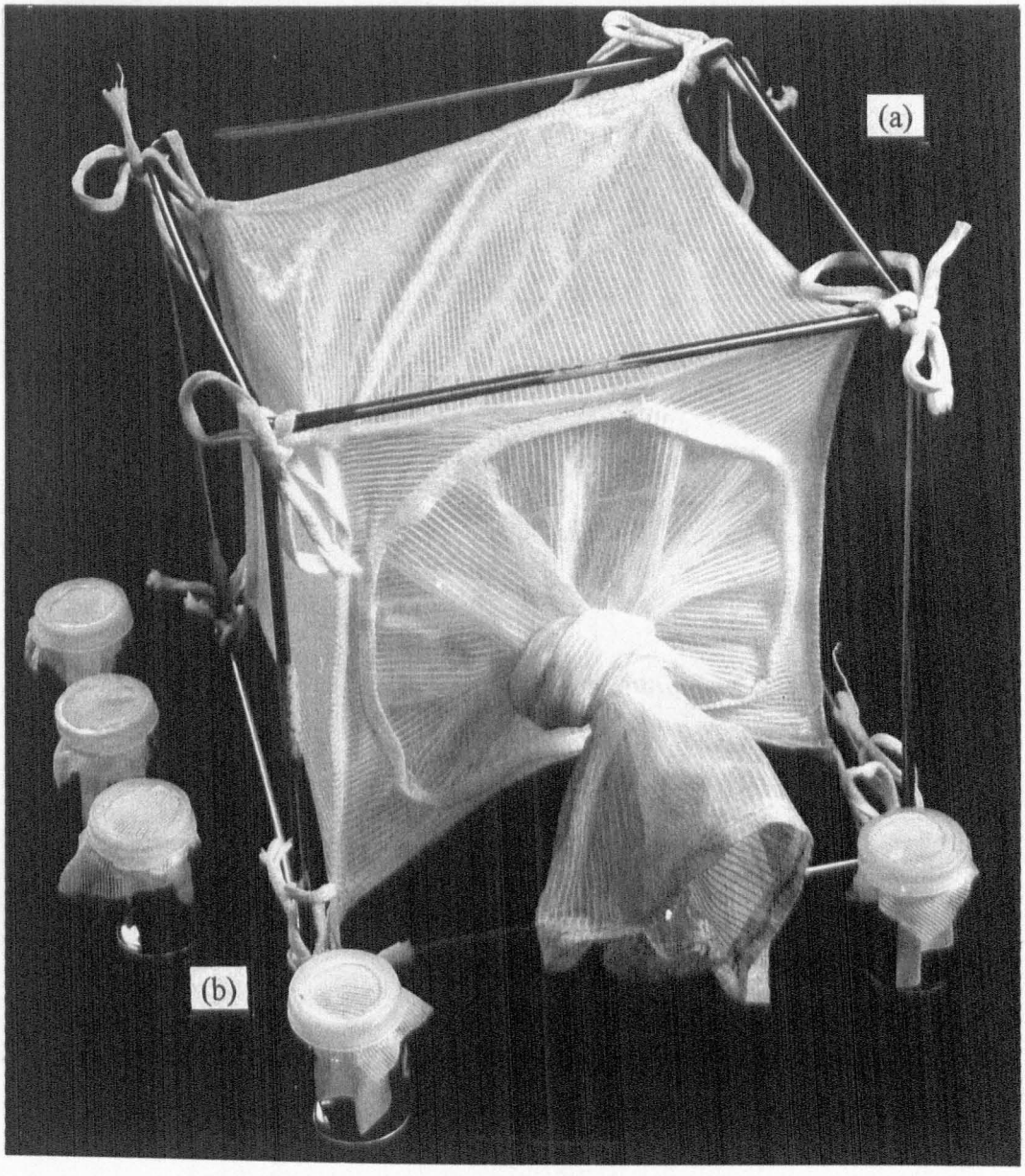


Figure 2.2. (a) Net cage used to enclose adults. (b) Oviposition tubes.

Figure 2.3. Hilton pot in moist, sand-lined plastic box, used in larval culturing.

Under these conditions in the UK, females laid an average (± s.e.) of 47.3 (±3.6) eggs (n = 50 females) and retained 1.2 (±0.8) eggs. Development from female engorgement to adult emergence was approximately 5-6 weeks and on average (± s.e.) 23% (±5%) of egg laid emerged as adults. A comparison of the proportions of eggs emerging as adults in Brazil and the UK showed that offspring survival was similar under both culturing techniques ($t = 0.11$, $n = 20$ Petri dishes, NS). In both environments, populations were maintained at around 500 emerging adults per generation by regulating the number of females that were bloodfed. When virgin flies were required, flies were released into an empty cage at less than 12hr post eclosion, and prior to sexual maturity of the males (Chaniotis 1967). Following this they were sexed and transferred to single sex cages. Unless they were to be killed immediately, flies were not mechanically aspirated as this often damaged them (Killick-Kendrick *et al.* 1977).

Preliminary experiments

In the remainder of this chapter I describe a series of experiments that I carried out to collect baseline data on natural history and key techniques that are central to work described later in this thesis. Many of the statistical tests described in this chapter will be referred to in the remainder of the thesis. In general, parametric tests were performed in Excel 5.0 or GLIM 4.0, transforming the data to achieve normality where necessary. Where the distribution could not be normalised, non-parametric tests were either performed in Statview 4.0, or the data was analysed using a logistic model in GLIM 4.0 specifying an appropriate error structure (after Crawley 1993). A Poisson error distribution was specified when data were counts, a Binomial when data were proportions and a Gamma when the variance:mean ratio was approximately one. For all logistic models and parametric tests performed in GLIM 4.0, parameters were specified as either continuous variables or multi-level factors. The significance of each parameter was checked by stepwise deletion. Significant terms ($P < 0.05$) were added back into the model before removing the next parameter (after Crawley 1993). Non-significant terms

were not re-introduced. The estimated change in deviance was compared with values of χ^2 (for a logistic model) or F (for parametric tests) given in standard statistical tables. If the minimum adequate logistic model (the model containing only significant terms) was overdispersed, such that the residual deviance was greater than the degrees of freedom, it was corrected by multiplying by the scale parameter (after Crawley 1993). The resulting scaled change in deviance for each parameter was then compared with values given by an F-test. For those experiments where multiple comparisons were made on a single data set the predicted significance values were corrected for the numbers of comparisons made using a sequential Bonferroni test (Sokal and Rolfe 1995, pp 239-241). Uncorrected results are given throughout, but only values less than the specified adjusted 95% significance level are discussed. Sokal and Rolfe (1995) and Siegel and Castellan (1988) were used for all statistical tests, notation and P values.

(i) Density dependent survival

In nearly all the experiments described in this thesis larvae are maintained at similar densities by standardising the numbers of eggs introduced into culturing pots. Thus even if it occurs, density-dependent larval mortality is unlikely to differ between treatment groups. In Chapter 6, however, I aim to raise and compare the survival of offspring from different individually-known females. Because *L. longipalpis* females vary in the number of eggs that they lay (Killick-Kendrick *et al.* 1977, pers. obs.), there is thus a potential problem of density-dependent larval survival confounding the results. This first experiment therefore aimed to explore the relationship between immature offspring survival over successive instars and initial egg density.

Methods

A total of 81 adult females and a similar number of adult males were released within 12hr of eclosion into mixed-sex cages for 24hr. As females mate readily in the laboratory (pers. obs.) this period of time was considered sufficient for all of them to mate. All females were transferred to glass oviposition tubes, covered with netting (3cm high, 2cm diameter; Figure 2.2b). Each contained a

small piece of moist, folded filter paper that provided a resting place for flies and a preferred oviposition site for females (Elnaiem and Ward 1992). At 3-4d old, females were released in batches of 10-20 into net cages and bloodfed on an anaesthetised hamster (see above for a description of the technique). They were then returned to their individual tubes for oviposition and supplied daily with small swabs of cotton wool soaked in sugar solution.

Females were placed into one of two treatment groups. In the first group (multiple egg batches, $n = 49$ females), all eggs laid were counted and reared in densities of approximately 150-250 eggs per Petri-dish (10cm diameter, 1.5cm height), with a mean (± s.e.) of 5 (± 0.32) females contributing eggs to each dish. In the second group (single egg batches, $n = 12$ females), all the eggs laid by a single female were counted and then raised together in an individual Petri-dish, at a mean (± s.e.) density of 47.8 (±6.0) eggs per dish. After death the abdomens of all females were dissected at x20 magnification and any retained eggs counted. Petri-dishes were checked daily for egg hatch and small amounts of liver powder were added. The total numbers of larvae surviving successive instars, the total number of pupae and the numbers of males and females emerging as adults were counted. The proportion of larvae surviving successive instars from single and multiple egg batches was calculated. As data could not be successfully transformed to normality, comparisons were made using a non-parametric Mann-Whitney U-tests. Results were corrected for seven multiple comparisons (see above) giving an adjusted 95% significance level of $P < 0.007$. Decreases in sample size during the experiment were due to deaths of all the offspring in some dishes throughout the lifecycle.

Results

A total of 19 females died prior to oviposition. Of these, 26% (5/19) had eggs and all others had undigested blood in their abdomens and had not produced eggs prior to death. Ovipositing females (62/81) laid a mean (± s.e.) of 44.5 (± 4.1) eggs. Few ovipositing females retained eggs (8/62) and they did not retain significantly more than non-ovipositing females (mean [± s.e.] number of eggs

retained by ovipositing females = 0.19 [± 0.5], n = 61 females; non-ovipositing females = 2.16 [± 1.2], n = 19 females; Mann Whitney U-test: $Z = 0.97$, NS).

A comparison of larval survival between single and multiple egg batches revealed a significant difference at one stage of the immature lifecycle: a significantly higher proportion of 1st instar larvae emerged as 2nd instars when reared in multiple batches (Table 2.1). The overall proportion of eggs emerging to adults was, however, similar for both treatment groups (Table 2.1). Moreover, there was no correlation between the proportion of offspring surviving from eggs through to adulthood and the absolute numbers of eggs per dish (r_s = 0.02, n = 17, NS).

Table 2.1. Survival of offspring reared in single and multiple batches, from egg laying through to adult emergence. Uncorrected significance values are given by: NS = not significant, ** = $P < 0.01$.

Stage in immature lifecycle	Median proportion of larvae surviving (number of Petri dishes)		Mann-Whitney U-test
	Single female	Multiple females	
Egg-1st instar	0.48 (12)	0.48 (8)	Z = -0.39 NS
1st-2nd instar	0.76 (12)	0.95 (8)	Z = -2.65 **
2nd-3rd instar	0.95 (11)	1.0 (8)	Z = -1.70 NS
3rd-4th instar	1.0 (9)	0.87 (8)	Z = 1.89 NS
4th instar-pupae	1.0 (9)	1.0 (8)	Z = -1.17 NS
Pupae-adults	0.38 (8)	0.48 (8)	Z = -0.32 NS
Eggs to adults	0.13 (8)	0.14 (8)	Z = -0.32 NS

(ii) Marking of male flies

A pre-requisite for studying male reproductive success (Chapters 3, 5 and 7) is that individual males can be successfully identified. As *L. longipalpis* do not

bear any obvious distinctive markings or patterns which can be used for identification, it was thus necessary to mark them. Fluorescent powders were used for marking for two reasons. First, as individuals are relatively small, it is not practical to use heavier paints or remove parts of their bodies. Second, fluorescent powders have previously been used in other studies of sandflies (Dye *et al.* 1987, Pardo *et al.* 1996). In this experiment, I outline the protocol that I used for marking males and test the fundamental requirement of any marking technique that it "shall not affect the longevity or behaviour of the animal" (Southwood 1978).

Method

A total of 100 males were selected from single-sex cages and were placed individually in oviposition tubes for marking with fine fluorescent powders (Fiesta daylight fluorescent colours: H. Haeffner Ltd.) in one of four colours (orange, yellow, pink, green). Males were marked by blowing approximately 0.1ml of the powder from a syringe into the oviposition tube. The powder formed a fine cloud that covered males. Any males that were dusted so heavily that their mobility was reduced, or which spent long periods preening, were discarded from both these trials and all other experiments requiring marking. Control males, were treated in the same manner as marked males except no powder was added to the syringe. After treatment, males were placed in oviposition tubes, supplied with sugar on swabs of cotton wool and checked daily until death.

The mean numbers of days survived across treatment groups of all adult offspring were compared in GLIM 4.0 employing a Weibull distribution model to generate predicted survival curves (after Crawley, 1993). Traditional methods use an exponential model to calculate survival parameters, however this assumes that the probability of death is constant across all age groups, which is likely to be unrealistic. The Weibull distribution model provides a better approximation than the exponential distribution as it is a two parameter model that estimates the scale and age specific probability of death (given as α), of the survival curve (see Pinder *et. al.* 1978 for a full description of the model). If $\alpha > 1$ the risk of death increases with age, if $\alpha = 1$ the risk of death is constant with age and values of $\alpha < 1$ indicate

a decreasing risk of death with age. (These correspond to the standard Type I, Type II and Type III (see Pinder *et al.* 1978 and references therein) survivorship curves respectively.) To estimate the effect of marking the predicted value of α was incorporated as an additional parameter into a logistic model, with a Poisson error distribution and male colour as a factor with five levels. The significance of each parameter was checked by stepwise deletion (as above). Differences between treatment groups were analysed using the Tukey-Kramer method (Sokal and Rohlf 1995).

Results

I observed no obvious behavioural differences between males across treatment groups: all males were active and wing-fluttered in the tubes irrespective of marking. All males survived for at least six days. The logistic model predicted a significant difference in the average numbers of days survived across treatment groups ($\chi_4^2 = 12.29$, $P < 0.05$; Table 2.2). Pairwise comparisons of the numbers of days survived between treatment groups indicated that the only significant comparison was males marked with the yellow fluorescent powder survive less well than unmarked males (Tukey-Kramer method: $P < 0.05$).

Table 2.2. Effect of coloured fluorescent dyes on male survival. Mean days survival and standard errors were calculated from the predicted values given by the Weibull distribution model.

Treatment (n = 20 males)	Mean days to death	Standard error
Unmarked	11.9	1.3
Orange fluorescent powder	10.3	1.4
Green fluorescent powder	9.6	1.4
Pink fluorescent powder	10.7	1.4
Yellow fluorescent powder	9.2	1.4

(iii) Sperm depletion

In insects, male fertilisation ability has been shown to decline over several successive matings (Markow *et al.* 1978, Rutowski 1979). In the laboratory, male *L. longipalpis* mate with several females if given the opportunity (Jarvis and Rutledge 1992, pers. obs.), and experiments outlined in Chapters 5 and 6 involve rearing the offspring of males that have mated repeatedly. If males suffer from sperm depletion with successive copulations this could confound interpretation of these results. The aim of the third experiment was therefore to explore to what extent the number of matings a male had recently achieved affected a female's probability of fertilisation. The results from this experiment will be used as a guideline for the experimental design used in Chapter 6.

Method

Each of 17 virgin males held in net cages was sequentially presented with ten bloodfed virgin females, at 10min intervals. Pairs were left until they copulated and the females were then immediately placed into oviposition tubes. Females were checked daily for oviposition and death and supplied with cottonwool swabs soaked in sugar solution. After death, the abdomens of females were dissected for retained eggs. For each female, all eggs laid were transferred onto a Petri-dish lined with moistened filter paper and permitted to hatch. The Petri-dishes were checked daily and after the first eggs were seen to hatch the dishes were left for a further five days prior to counting the total number of 1st instar larvae present in the dish.

One male failed to fertilise any eggs and was excluded from the analyses. For the remaining males, the length of copulation; the number of eggs laid and retained by a female were compared with female mating order. The number of eggs which hatched per female were also compared using a logistic regression, assuming a Binomial error distribution. The numbers of eggs laid was set as the binomial denominator and female mating order and male identity were added as a continuous variable and a factor with 16 levels respectively. The significance of each parameter was assessed as above.

Results

The proportion of eggs laid that then hatched varied significantly with both female mating order ($\chi_1^2 = 259$, $P < 0.001$, Figure 2.4), and male identity ($\chi_1^2 = 463.8$, $P < 0.0001$). The data suggested visually that the proportion of eggs hatching was comparable across the first six females and only declined for the seventh and subsequent females. To test this I compared the difference in the residual deviance of two models. In the first model I included female mating order as a continuous variable and in the second model I combined the data for the first six females and compared this with the combined results for females mated in positions seven to ten. There was no significant difference in the residual deviance between the two models ($F_{1,158} = 0.86$, NS), indicating that combining the data for the first six females was justified. Two further logistic models, comparing the proportion of eggs hatching with female mating order, confirmed that there were no differences in the proportion of eggs hatched from females mated in positions one to six ($\chi_1^2 = 1.13$, $n = 96$, NS), or positions seven to ten ($\chi_1^2 = 1.9$, $n = 64$, NS).

The length of time spent in *copula* was comparable across all females (geometric mean [± s.e.] = 45.8sec [±1.7]; $F_{1,159} = 3.75$, NS). The mean numbers of eggs laid and retained by females also did not vary with the order in which females were presented to males (mean [± s.e.] number of eggs laid = 16.8 [±5.0]; $F_{1,159} = 0.17$, NS; geometric mean number retained [± s.e.] = 1.9 [±3.6]; $F_{1,159} = 0.71$, NS).

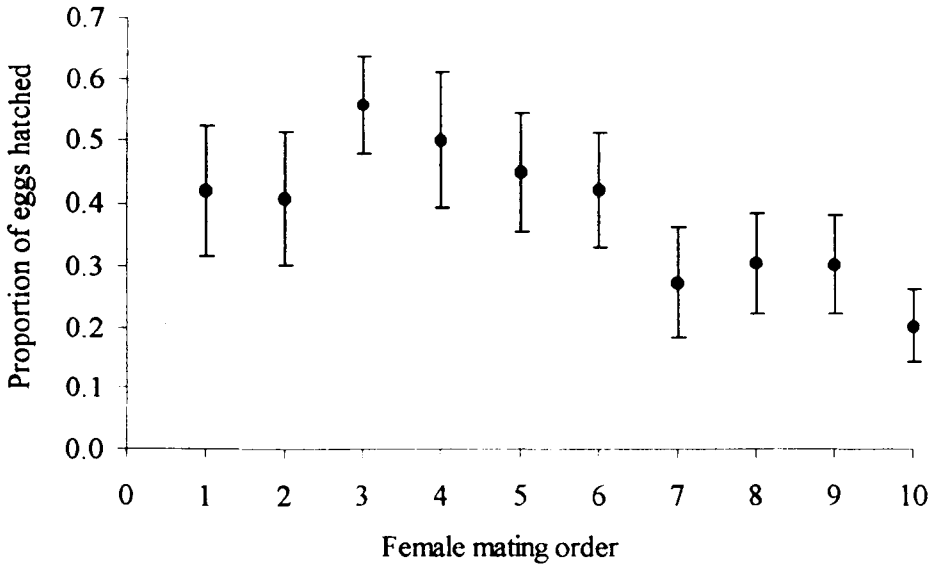


Figure 2.4. Relationship between mean (±s.e) proportion of eggs hatched and female mating order.

(iv) Multiple mating by females

Multiple mating by females is common in insects and introduces the possibility that sperm competition may be an important determinant of male reproductive success (Simmons *et al.* 1993, Siva-Jothy and Hooper 1995; for discussion see Simmons and Parker 1996). In the field, *L. longipalpis* females have the opportunity to mate with several males per reproductive cycle. In the laboratory, however, females are rarely observed to mate for a second time immediately after mating (pers. obs.). The aim of the final experiment in this chapter was to quantify the extent to which laboratory females will, if given the opportunity, mate multiply within a single gonotrophic cycle. Mated females were re-exposed to male aggregations 1hr, 24hr, or 72hr after their first mating. These time periods were chosen to represent a female that had mated and remained in an aggregation; a mated female that returned to an aggregation the following evening, and the maximum period between the first bloodfeed and oviposition respectively.

Method

Three groups of 30 virgin, bloodfed females were introduced individually into net cages with ten males and allowed to mate once. Females were observed continually until they copulated, the duration of copulation recorded and the female then removed. After this all females were placed in oviposition tubes for one hour and then introduced to a fresh set of ten males after 1hr, 24hr or 72hr. Females were then observed for a further hour and data on copulation and behaviour were collected as above. The length of first matings and second matings (where they occurred) were compared across the three groups of females. Data could not be transformed to normality so were analysed using non-parametric tests.

Results

The length of time spent in *copula* in the first trials was comparable for all three groups of females (Table 2.3). Only 18% (16/90) of females remated, and these matings were significantly shorter than first matings of females (Mann-Whitney U-test: $Z = 4.23$, $P < 0.0001$, $n = 16$ second matings). No female was seen to copulate more than twice. Behaviourally, virgin females appeared to solicit copulations actively: they were observed to wing-flutter and approach several males prior to copulation. Mated females appeared less active irrespective of the time interval between their first and second exposure to males. They remained motionless in the cage and moved away from approaching, wing-fluttering males. Few females mated for a second time and only one of those that mated did not depress her abdomen or pull away from the male thereby actively terminating the copulation attempt. This female had previously terminated her first mating, suggesting that her first copulation was unsuccessful.

Table 2.3. Comparison of copulation lengths and the incidence of remating across females permitted second matings after three time intervals (1hr, 24hr, 72hr). Significance level given by: NS = not significant, n = 30 females in each group.

	Treatment group			Kruskal Wallis
	1hr	24hr	72hr	(H_{corr})
Median length of 1st copulation (sec)	37.5	38	35	4.70 NS
Number of 2nd copulations	8	8	0	-
Median length of 2nd copulations (sec)	2.0	3.0	-	-

Discussion

The most important results from this series of experiments are as follows. Offspring survival from egg through to adult was not density dependent, thus survival of offspring from individual females is not likely to be confounded by the effects of rearing density. The use of fluorescent powders to mark males did not impair their movement and while yellow fluorescent powder had a negative effect on male survival, all males survived longer than 6 days. Males successfully fertilised up to six females without suffering from sperm depletion, suggesting that the offspring of females mated in sixth position or less may be reared without differences in male fertility confounding interpretation of survival. Mated females were reluctant to remate in a single gonotrophic cycle, irrespective of the time elapsed since their first mating suggesting that sperm competition may not be an important determinant of male reproductive success in *L. longipalpis*. This results also indicates that while females are exposed to several males in the field, permitting them a single mating in the laboratory may not be completely unnatural.

Chapter 3

Does *Lutzomyia longipalpis* lek?

Summary

1. In Bradbury's (1981, 1985) definition of a classical lek-breeding species, males aggregate at sites (or leks), where they defend territories against other males. Females visit these sites to copulate and can select their mate freely. Males are emancipated from parental care, and do not defend resources required by females.
2. A previous study proposed that in one population of the sandfly *Lutzomyia longipalpis*, males form lek-like aggregations in the laboratory (Jarvis and Rutledge 1992). Here, I describe a series of experiments which aim to test whether the *L. longipalpis* population, from Marajó Island, Brazil, satisfies the criteria of lek-breeding. Specifically, the following questions were addressed: do males aggregate and perform agonistic displays in the laboratory and field? Is a host (a resource) necessary for mating, and does the presence of a host affect the distribution of males and matings? Finally, are females free to choose their mates?
3. The experiments confirmed that the mating aggregations of Marajó sandflies constituted leks. In chicken sheds in the field, males formed aggregations on or near hosts. In the laboratory, males formed stable aggregations irrespective of host presence. Agonistic behaviour was observed between males in both the laboratory and field.
4. Although, males aggregated on or near hosts, laboratory evidence shows that a male on a host was no more likely to obtain a mating than a male in other regions of the cage. Furthermore, a host does not appear to be necessary for a female to accept a mating: females mated in these aggregations as readily as when a host was absent or when it was present. Male mating success was significantly skewed such that a few males obtained the majority of available matings. The data indicated that this observed variance in male reproductive success was at least partially determined by female choice: females were free to reject males both prior to and after genital contact.
5. Differences in the results obtained in this study and the previous laboratory study on another population of *L. longipalpis* are discussed.

Introduction

Lek-breeding is defined as occurring where territorial males form aggregations (or leks) which contain no appreciable resources for females, where males are emancipated from parental care, and where females visiting male aggregations can choose their mates freely (Bradbury 1981). Despite the fact that by definition, lekking females do not gain direct benefits such as enhanced access to resources or care for their offspring from their choice of mate, they exhibit considerable unanimity in mating preference and male mating success is typically highly skewed so that a small number of males obtain a large proportion of the matings (Bradbury *et al.* 1985, Wiley 1991, Höglund and Alatalo 1995).

Lekking has been reported in a diverse array of mammals, birds, fish, amphibians, and insects (for reviews see Baker 1983, Alcock 1987, Wiley 1991, Andersson 1994, Höglund and Alatalo 1995). While it is generally agreed that lekking males do not defend resources or contribute to parental care, there is continuing debate about the criteria used to distinguish lek-breeding. For example, variation in the degree of male aggregation has prompted the term "exploded lek" to account for those leks where males are somewhat more dispersed than in the classical definition of a lek (Emlen and Oring 1977, Bradbury 1985). In addition, in some lek-breeding species, it is unclear how far a female is free to choose a mate (Bradbury 1985, Trail 1985, Beehler and Foster 1988, Droney 1992; see below). Moreover, even where females can choose their mates freely, it has been suggested that mate choice copying may exaggerate the variance in male mating success observed in many lek mating systems (Wade and Pruett-Jones 1990, Dugatkin 1992, Gibson and Höglund 1992, Clutton-Brock and McComb 1992, McComb and Clutton-Brock 1994, Kirkpatrick and Dugatkin 1994). Finally, it has also been proposed that, even on a lek, a female could gain immediate benefits from her choice of mate, through reduced risk of predation, reduced risk of contracting sexually transmitted diseases, reduced mating harassment

by undesirable males, or increased probability of fertilisation (Halliday 1978, Wrangham 1980, Reynolds and Gross 1990, Kirkpatrick and Ryan 1991, Wiley 1991).

Departures from leks *sensu strictu* are perhaps most apparent in insects, where species exhibiting lek-like mating systems often do not conform to the strict lekking criteria proposed initially for mammals and birds (Baker 1983, Alcock 1987, Höglund and Alatalo 1995). Thus while territorial aggregation at particular sites has been reported for a variety of insect species (Alcock 1981, Otronen 1984a, 1984b, Wickman 1985, Shelley 1987, Hoffman 1987, Hoffman 1988, see also Baker 1983 for a review), there remains considerable debate about the extent to which visiting females are free to choose their mates. For example, there is little evidence for female choice in the mating aggregations of swarming insects, although they fulfil all other criteria of lek-breeding: males do not contribute to parental care and aggregate at non-resource based sites which females visit primarily to copulate (Downes 1969, Allan and Flecker 1989, Neems *et al.* 1990, 1992, Harker 1992, see also Thornhill and Alcock 1983 for a review). An additional subject of controversy is that, in many insect species, males aggregate and defend sites close to resources used by females (Campenella and Wolf 1974, Parsons 1977, Parsons and Bock 1977, Baker 1983, Otronen 1984, Bradbury 1985, Alcock 1987, Hoffman and Blows 1992, Jarvis and Rutledge 1992). For example, males of *Drosophila mycetophaga*, aggregate and defend small territories on the underside of bracket fungi, which females visit to copulate (Parsons 1977, Parsons and Bock 1977). Although females use the fungal exudates as oviposition sites (Hoffman and Blows 1992), aggregations of males were not more common on those fungi which contained resources than those without available resources. Moreover a male's access to a resource did not determine his mating success (Aspi and Hoffman 1995). These concerns led Bradbury (1985) to note that many species satisfy some, but not all, of the necessary criteria for classic lek formation and that the diversity of lek-like mating systems was also worthy of exploration (for discussions, see Alcock 1987, Höglund and Alatalo 1995). In the same paper, Bradbury also explicitly states that it is not the absence of resources within a territory which should distinguish

lekking and non-lekking species, but whether a female's differential access to a resource determines a male's mating success.

Several species of phlebotomine sandflies form male-biased aggregations on the buttresses of trees (Memmott 1991, 1992, Memmott and Sutton 1994) or on bloodmeal sources (Lane *et al.* 1989, Jarvis and Rutledge 1992). Males of the *Lutzomyia longipalpis* species complex aggregate on or near hosts in chicken sheds (Dye *et al.* 1991) and in the laboratory have also been observed to aggregate on the backs of anaesthetised mice or hamsters (Jarvis and Rutledge 1992). Females visit hosts to mate and to obtain a bloodmeal necessary for egg development, but neither sex contributes to parental care. Females mate on aggregations, feed nearby and then oviposit their eggs after a three day period of maturation (although note that oviposition sites have not yet been located in the field - see Chapter 2). In a recent study, the mating behaviour of *L. longipalpis* was investigated in more detail, using a small cage in a laboratory environment (Jarvis and Rutledge 1992). Males were observed to aggregate on or around hosts, where they defended small territories from approaching males. The formation of an aggregation was partially age-dependent: older males were more likely to form aggregations on hosts than younger or middle-aged males. Removal of a territorial male from the host resulted in immediate occupation of the vacant territory by a previously non-territorial male. Matings or attempted matings were more likely to occur in the lower half of the cage and specifically on the host, although females appeared generally reluctant to mate. Based on these observations, the authors concluded that the mating behaviour of *L. longipalpis* resembles that of lekking species (Jarvis and Rutledge 1992). There are however several problems with such an interpretation. Most importantly Jarvis and Rutledge fail to provide necessary evidence for two key criteria for lek-breeding. They do not explore whether females are free to choose their mates. Moreover, their results suggest that male territories contain resources needed by females, and do not rule out the possibility that male mating success may be determined by the quality of those resources.

Here I describe a set of experiments aimed at re-examining the mating system of *L. longipalpis* using a population collected from Marajó, Brazil, and reared for approximately 20 generations in the laboratory. It is known that the Marajó population has a different pheromone type and is possibly a different sibling species (G. Hamilton pers. com.) to the Minas Gerais (Brazil) population used by Jarvis and Rutledge (1992). The study was designed to explore whether *L. longipalpis* satisfy the criteria necessary for a lekking species as defined by Bradbury (1981, 1985). Specifically the following questions were addressed: do males aggregate in specific sites in the field and in the laboratory? Do males display agonistic behaviour in the field and laboratory? Is a host necessary for mating to occur and does the presence of a host affect the distribution of males and matings? To what extent are females free to choose their mates? Finally, I discuss possible behavioural differences in the mating systems of the two populations of *L. longipalpis*.

Methods

All flies used in laboratory experiments were 4-6d old virgins released into single-sex stock cages less than 12hr after adult emergence. The age of flies observed in the field was unknown.

(i) Male aggregation and aggression

To assess the degree of aggregation and temporal stability of male location, I investigated the distributions both of freely dispersed males in chicken sheds in the field and of laboratory males held in small cages. Levels of male aggression were assessed by observing male behaviour on chickens (hosts) in sheds in the field and laboratory.

Field study

In order to explore the spatial and temporal distribution of flies in the field, observations were carried out in two chicken sheds in the village of Pingo d'Água, Marajó, Brazil (48°31'S, 0°46'W). The sheds were approximately 200m apart, and were known to attract large numbers of flies (pers. obs.). Shed 1 contained six chickens, and shed 2 three chickens. Flies were free to distribute themselves across all regions in a shed. Observations took place between 3 August and 28 October 1995. The sheds were visited for up to eight evenings at 15min intervals between 19:00hr and 20:45hr. At each visit, the numbers of males and females present on individual chickens, <1m from chickens and >1m from chickens were recorded. These three regions were defined as host, close and distant respectively. Observations were made by torchlight for approximately 5min in each shed. To provide an account of male aggression in the field, the behaviour of males present within chicken sheds was noted while counting the numbers of flies present. Males were defined as exhibiting agonistic behaviour if they were observed to wing-flutter at approaching males, engage in fights in which they tumbled over one another, or if they clashed abdomens with each other.

As chickens were not individually identified and often changed places within a shed between, but not within nights, each night the chickens in a shed were ranked with respect to the total numbers of flies they supported during that evening. Thus, within a night, the highest ranked host was always host 1 and the lowest ranked host was always host 6 (shed 1) or host 3 (shed 2). All chickens were approximately equal in size. Average numbers of males in each region were compared using logistic regressions, assuming a Poisson error distribution. Date and region in the shed were added to the model as factors. The model for shed 1 was corrected for overdispersion and the significance of each parameter was determined as described in Chapter 2. To assess whether the distribution of males across chickens was consistently aggregated in a shed, variance:mean ratios of the numbers of males on individual chickens were calculated for each observation period throughout a night. These were then compared with an expected random distribution (where variance:mean = 1) using a *t*-test and

values of *t* greater than the 95% significance levels found in tables were considered to have arisen from a significantly aggregated distribution. Finally, for each shed and night, temporal stability was explored by calculating Spearman rank correlations for the distribution of males across hosts in each shed at time t_0 (19:00hr) to the distribution of males at each successive observation period between 19:00hr and 20:45hr. If male distribution was temporally stable, it would be expected that the numbers of male flies observed on each host would be positively correlated.

Laboratory study

To explore whether males formed stable aggregations in a laboratory environment, groups of males were introduced and permitted to disperse freely in small cages. Eight sets of 10 virgin males were introduced into net cages (30x30x30cm). In half of these, an anaesthetised mouse (the host) was placed on the base of the cage prior to the start of the experiment. These trials were defined as "host trials"; trials run in the absence of a host were defined as "non-host trials". Males were observed for a 10min prior to the introduction of five virgin females; observations then continued for a further 20min period. The interior of the cage was divided into eight equal regions: the four quadrants, of the upper and lower halves of the cage. In host trials, a 9th region, the "host region" was defined as any area on the host or within 2cm from the body of the host. The distribution of the 10 males across the eight or nine regions of the cage was noted every minute throughout the experiment. The location and timing of all matings and the incidence of female feeding were also recorded. A subjective description of male behaviour (as described above) in the laboratory was also noted. All trials were carried out in the UK.

In host trials, I standardised the numbers of males observed using the surface area of the region prior to analysis. The surface area of the host region was calculated as approximately 195cm^2, (the sum of the estimated surface area of the host and the area within 2cm of its body); non-host regions in the upper and lower halves of cage

were 675cm^2 and 662.5cm^2 respectively. (Note that the lower halves of the cage all contained part of the host region.) The distribution and temporal stability of males were assessed in the same manner as the shed data, by calculating variance:mean ratios and correlation coefficients. As the distribution of males may not be stable immediately after their introduction and males may become disturbed upon introduction of females into a cage, t_0 was defined first as 5min prior to and then 5min after the introduction of females respectively. Thus five correlations were calculated in the absence of females (between 5min and 10min) and 15 (between 15min and 30min) in the presence of females.

(ii) The importance of resources

If the mating success of a male sandfly depends on his ability to defend resources, it would be expected that males would aggregate on or near hosts; females would mate where they feed; and in the absence of a host, females would be reluctant to mate. These predictions were tested using data from the host and non-host trials conducted in the laboratory as described above, standardising for differences in surface area.

To assess whether males preferentially aggregated near a host, I compared the proportion of males in the host and non-host regions and in the upper and lower halves of the cage, both 5min before and 5min after the introduction of females using a logistic regression. Data were standardised for differences in surface area prior to analysis. Because data were proportional a Binomial error distribution was specified. Trial and female presence were added to the model as factors with 4 and 2 levels respectively. The significance of each parameter was calculated as described in Chapter 2. I then compared differences in the location of males, of matings, and the latency period prior to the onset of matings across host and non-host treatments.

(iii) Female choice

Variance in male mating success

To explore the distribution of matings between males, 10 sets of five males were individually marked using fluorescent dyes (see Chapter 2) and introduced into net cages. Males were left for 10min before sequential introduction of 10 virgin females. All females were observed until they mated and then replaced by the next female until each set of males had been exposed to 10 females. For each female the identity of her mate was noted. To investigate the impact of female feeding status on the distribution of matings, an identical set of ten trials was run using virgin females which had been fed 24hr earlier. Ten further trials were run using smaller leks consisting of three males and a total of six fed females to explore the effect of lek size on the distribution of matings. Thus for all trials, mean male mating success is two copulations. The frequency distributions of matings were compared with those generated by computer simulations in which 10 matings were allocated randomly across 5 or 3 males, over 1,000 trials. All trials were run in the UK.

Role of female choice

If a female is free to choose her mate, she should be able to sample and reject males prior to copulation and avoid unwanted matings. The ability of females to choose freely was assessed by introducing them individually to laboratory aggregations of 10 males (as above), and then monitoring all their interactions with males in detail. This was carried out for 30 females (each with a different set of males). The outcome of all male/female interactions was recorded (for definitions see Table 3.1).

Table 3.1. Classification of male/female interactions according to their outcome and the behaviour of the female.

Female behaviour	Is interaction successful?
Female moves away from male as he approaches	No
Depresses abdomen as male attempts copulation	No
Wing-flutters at approaching male	Yes
Females motionless on male's approach	Yes

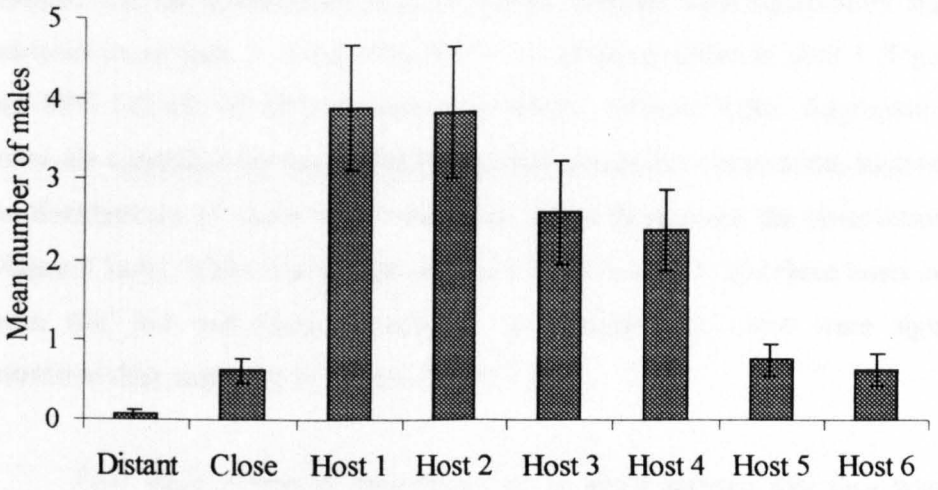
Results

(i) Male aggregation and aggression

Field study

Only four female flies were observed during the entire observation period and thus these were excluded from analyses. Males were observed for a total of eight nights in shed 1 and seven nights in shed 2. There were significant differences in the mean numbers of males present across the different regions in both sheds (effect of region on the mean numbers of flies observed in shed 1: $F_{7,55} = 5.43$, $P < 0.01$; shed 2: $\chi_4^2 = 43.31$, $P < 0.001$; Figure 3.1a-b). The majority of males were found displaying on a few of the available chickens, while the remaining flies distributed themselves at sites either on or near the other chickens. There were few observations of flies greater than a metre away from a chicken. The average numbers of males present varied with the night of sampling in shed 1, but not in shed 2 (shed 1: $F_{7,55} = 3.58$, $P < 0.05$; shed 2: $\chi_6^2 = 5.35$, NS). There was no significant interaction between date and region for either shed (shed 1: $F_{48,55} = 1.9$, NS; shed 2: $\chi_{24}^2 = 10.64$, NS).

(a) Shed 1



(b) Shed 2

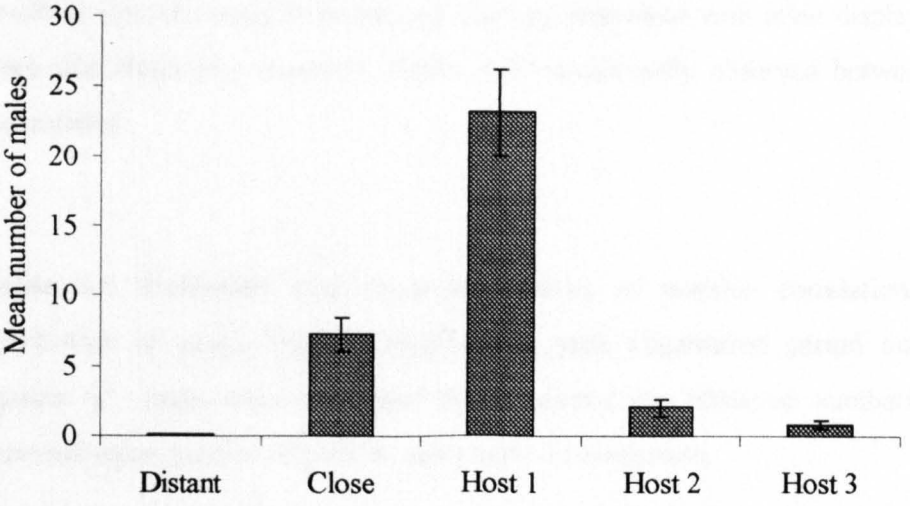


Figure 3.1. Mean (± s.e.) numbers of males observed in each region in (a) shed 1 - eight nights; (b) shed 2 - seven nights.

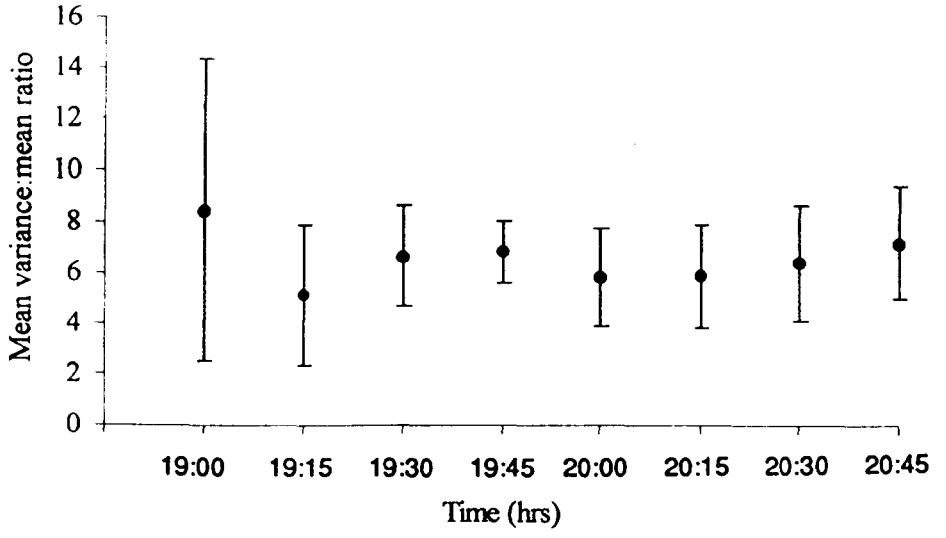
Restricting analyses to those males observed on chickens, variance:mean ratios revealed that the distributions of males across chickens were significantly aggregated (variance:mean ratio > 1) for 65% (33/51) of all observations in shed 1 (Figure 3.2a) and 86% (42/49) of all observations in shed 2 (Figure 3.2b). Aggregations were positively correlated between 19:00hr and each successive observation, suggesting that the distributions of males were temporally stable throughout the observation period (Figure 3.3a-b). While low sample sizes (six hosts in shed 1, and three hosts in shed 2) mean that few individual correlations were significant, more were significantly correlated than expected by chance (Table 3.2).

Field observations of male behaviour in sheds showed that they were active and exhibited agonistic behaviour. Males were jostled for space and wing-fluttered at approaching males. Agonistic encounters between males, which predominantly involved vigorous wing-fluttering and clashing abdomens with other displaying males were also frequently observed. Fights were occasionally observed between two or more males.

Table 3.2. Probability that observed numbers of positive correlations for the distribution of males between 19:00hr and each observation period occurred by chance. χ^2 values were calculated by comparing the observed numbers with an expected equal number of positive and negative correlations.

Shed	Number of correlated correlation coefficients		χ^2	*P* - value
	Positive	Negative		
1	53	1	30.1	< 0.001
2	49	0	49.0	< 0.001

(a) Shed 1



(b) Shed 2

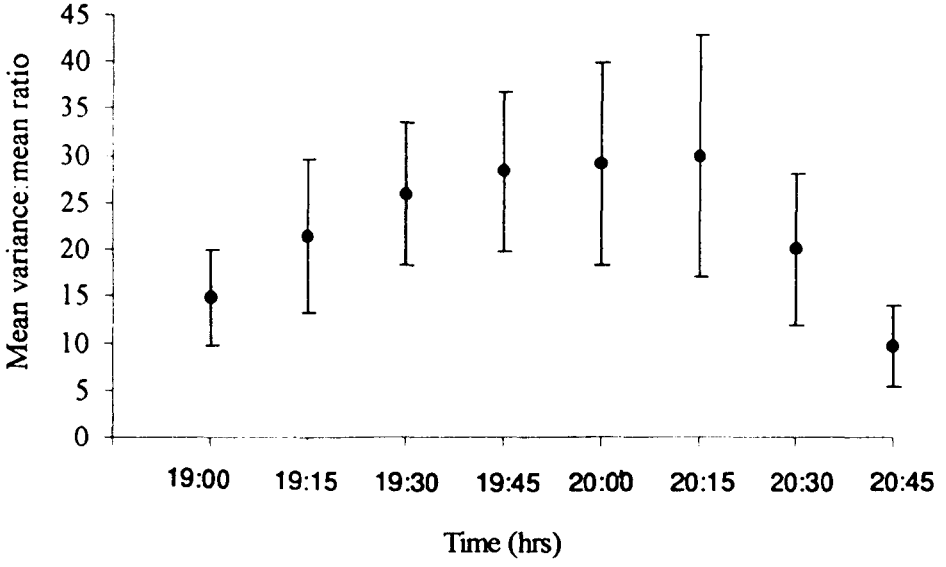
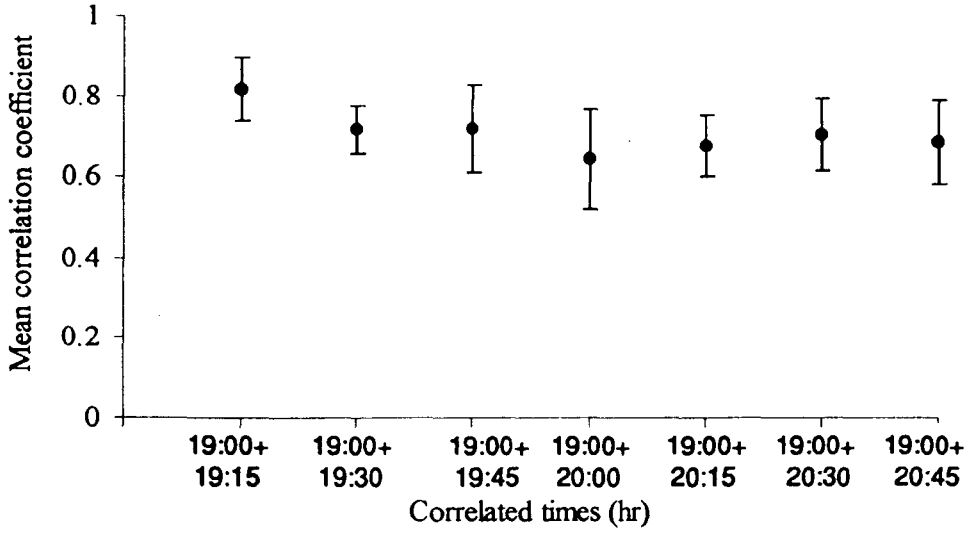


Figure 3.2. Mean (± s.e.) variance:mean ratios for distribution of males across the six hosts in (a) shed 1 - data are from 54 observations made over eight nights; (b) shed 2 - data are from 49 observations made over seven nights.

(a) Shed 1



(b) Shed 2

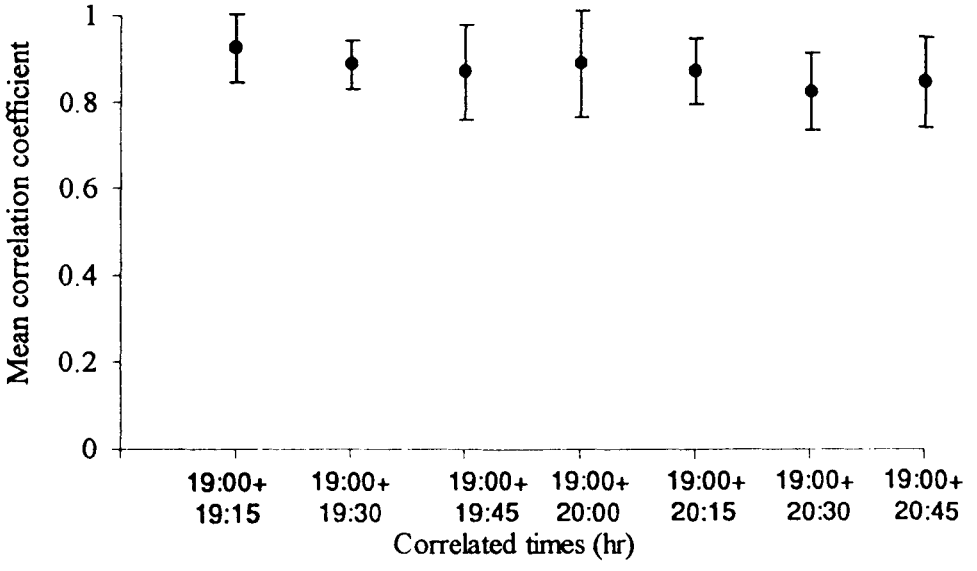


Figure 3.3. Correlation coefficients between the number of males on each host at 19:00hr and each successive 15min observation from 19:00-20:45hr. Points give the mean values across all nights; error bars show standard errors. (a) Shed 1 - eight nights; (b) Shed 2 - seven nights.

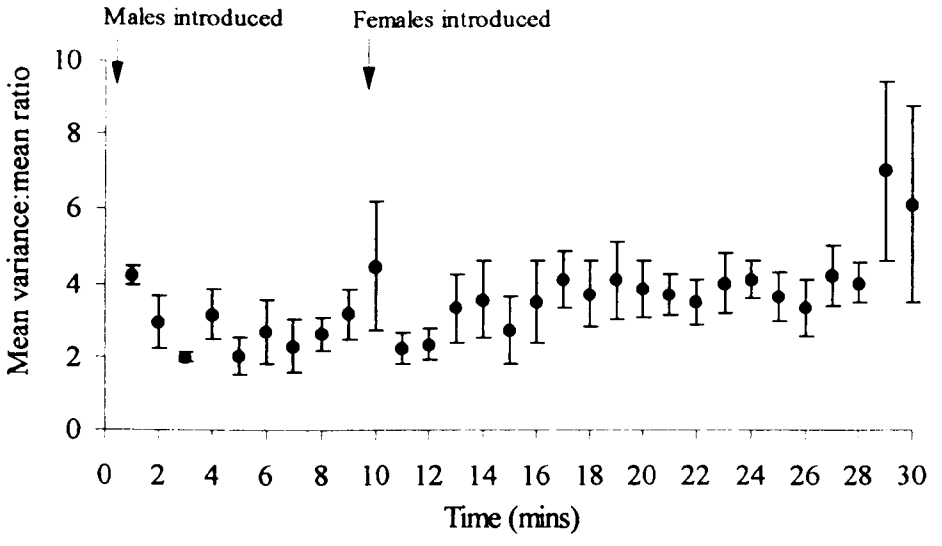
Laboratory study

In both host and non-host trials, the majority of males occupied 3 or 4 of the available regions in a cage. The distribution of males within a cage was significantly aggregated for 62% (74/120) of observations in non-host trials (Figure 3.4a) and 78% of observations in host trials (Figure 3.4b). In both sets of trials, the number of males present within a region was generally positively correlated between t_0 and subsequent observations, prior to and after the introduction of females (Figure 3.5a-b) and this was more frequent than expected by chance (Table 3.3).

Table 3.3. Probability that observed numbers of positive correlations for the distribution of males between 5min and each subsequent observation to 10min, and 15min and each subsequent observation to 30min occurred by chance. χ^2 values were calculated as in Table 3.2.

	Number of correlated correlation coefficients		χ^2	*P*-value
	Positive	Negative		
Non-host trials				
5min-10min	19	1	16.2	<0.001
15min-30min	60	0	60.0	<0.001
Host trials				
5min-10min	20	0	20.0	<0.001
15min-30min	59	1	56.1	<0.001

(a) Non-host trials



(b) Host trials

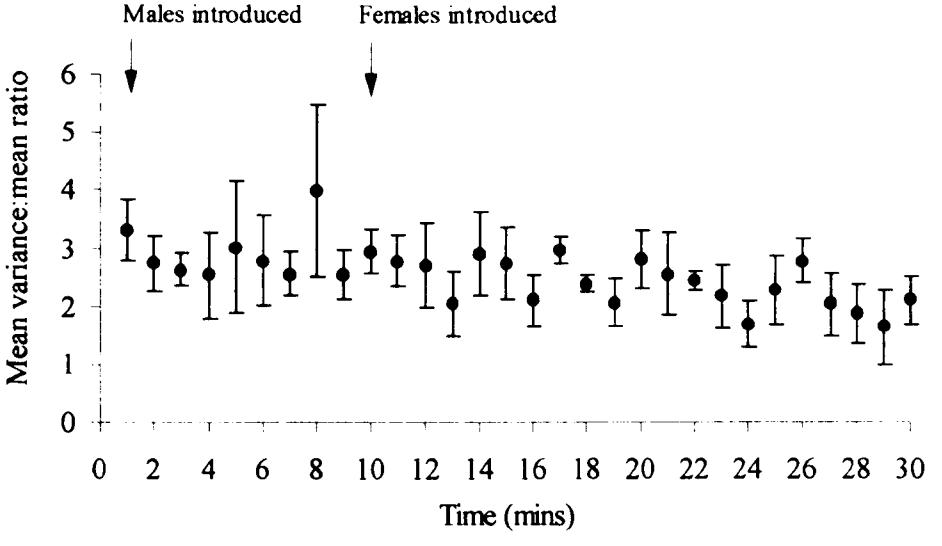
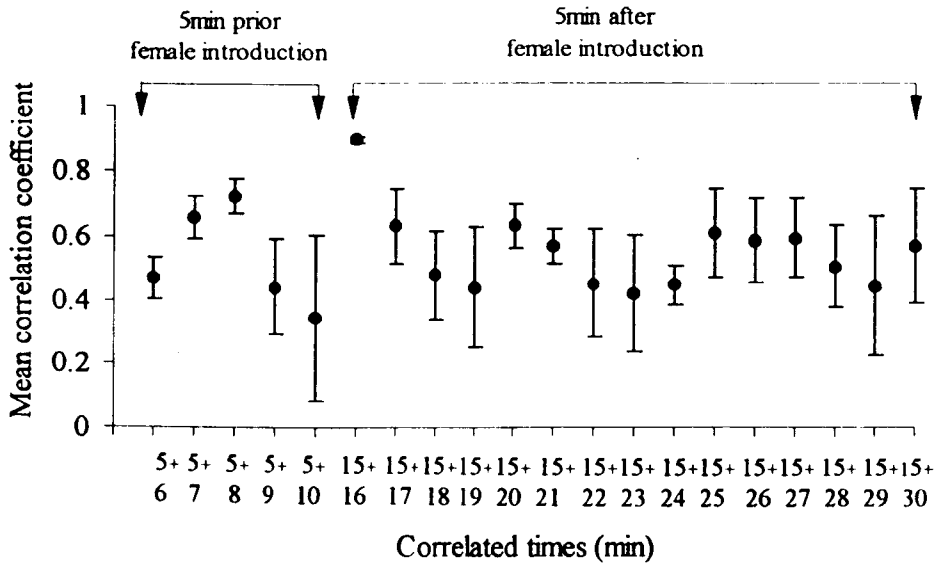


Figure 3.4. Mean (± s.e.) variance:mean ratios for distribution of male flies across available regions over a 30min observation period. Observations came from 4 trials run in (a) the absence of a host; (b) the presence of a host.

(a) Non-host trials



(b) Host trials

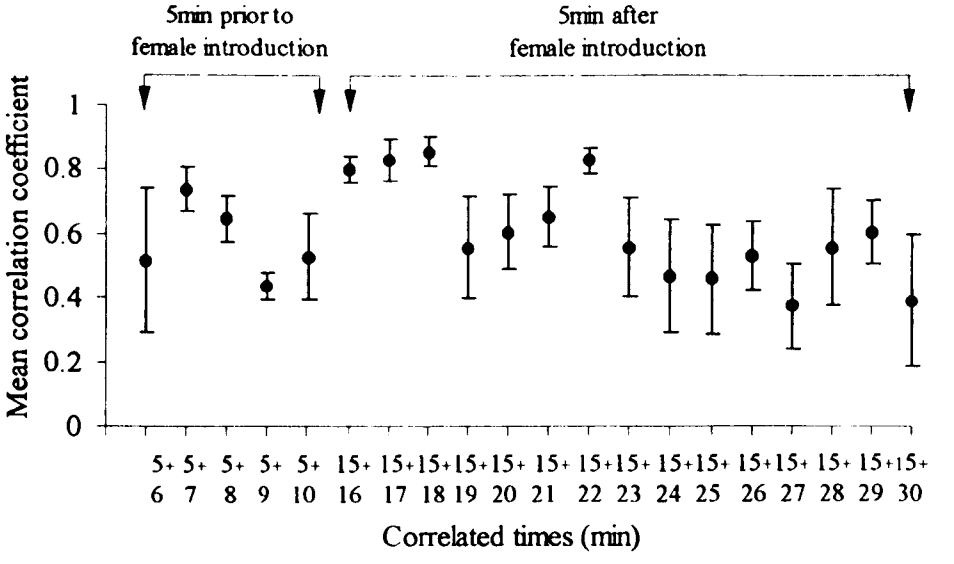


Figure 3.5. Correlation coefficients for distribution of males within a cage between 5min and each subsequent observation to 10min, and 15min and each subsequent observation to 30min. Data come from 4 trials each of 30min run in (a) the absence of a host; (b) the presence of a host.

Observations in host and non-host trials showed that male behaviour in small cage environments was comparable to that observed in the field trials. Irrespective of female presence, males wing-fluttered at approaching males and occasionally engaged in agonistic encounters which involved vigorous wing-fluttering and clashing abdomens, as observed in the field (see above). Occasionally fights between two males were observed. These involved males tumbling over one another and were usually resolved rapidly, resulting in one male leaving the contest site.

(ii) Importance of resources

Few males landed on the host in the four host trials (mean [± s.e.] number of males present on host = 0.20 [± 0.05]). When a male landed in the host region he generally stayed there for the duration of the 30min observation period. Restricting analyses to those observations made at 5 and 15min, and standardising for differences in surface area, males were not more likely to aggregate near the host than in other sections of the cage (mean [± s.e.] number of males in non-host regions = 1.03 [±1.0]; host region = 1.06 [±0.80]: $\chi_1^2 = 3.18$, NS), irrespective of female presence ($\chi_1^2 = 0.03$, NS) and trial ($\chi_3^2 = 0.02$, NS). While males tended to aggregate in the lower half of the cage in host trials, males were also equally likely to aggregate in the lower half of the cage in the absence of a host (2x2 chi-squared test with correction: $\chi_1^2 = 0.155$, NS; Figure 3.6).

All females mated within 10min of being introduced into the cage and the presence of a host had no effect on the latency period prior to mating (geometric mean time to copulation: non-host = 4.8min; host = 4.1min; $F_{1,37} = 0.47$, NS). When the host was present, 95% (19/20) of all matings occurred in regions away from the host. All matings occurred prior to feeding. In the single case where a mating occurred on the back of the host, the female bloodfed in a separate location away from her mate after copulating. When comparing the locations of matings across host and non-host trials, more matings occurred in the lower half of the cage, but there was no difference

between the distribution of matings in host and non-host trials (comparison of the number of matings in the upper and lower halves of the cage in host and non-host trials: Mann Whitney U-test: $Z = 1.78$, $P = 0.08$, $n = 8$, Table 3.4).

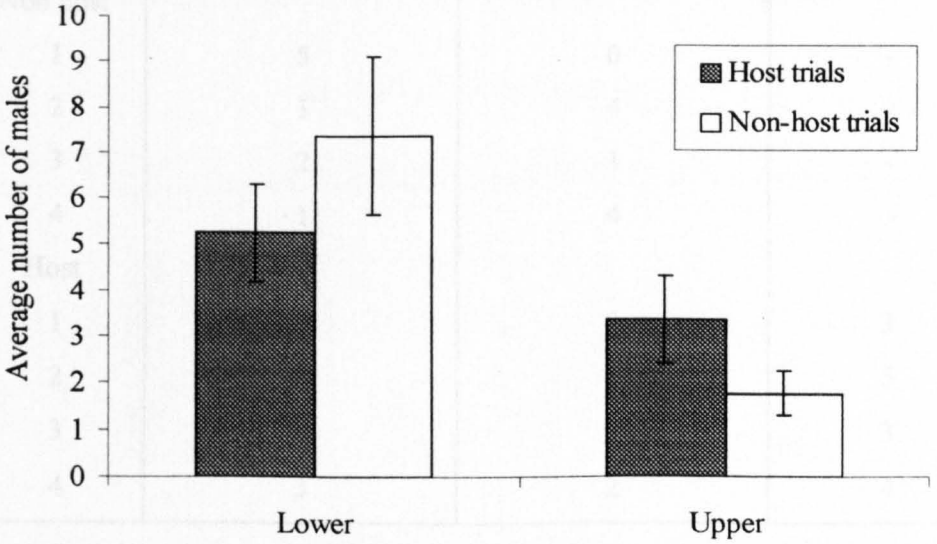


Figure 3.6. Mean (± s.e.) number of males observed in the upper and lower halves of the cage in host and non-host trials. Means were calculated from the results of two sets of trials: four in the presence and four in the absence of a host.

Table 3.4. Number and locations of matings and bloodfeeds across trials in the presence and absence of a host.

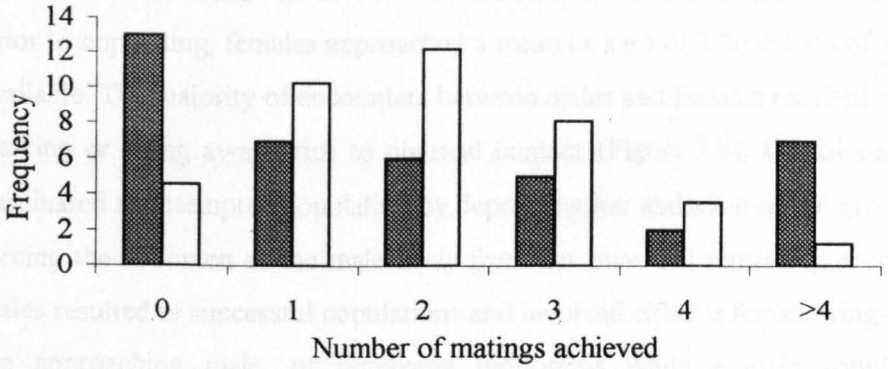
Trial	Total number of matings (out of 5 females)		Number of females feeding
	Upper cage	Lower cage	
Non host			
1	5	0	-
2	1	4	-
3	2	1	-
4	1	4	-
Host			
1	0	5	3
2	0	5	5
3	0	5	3
4	3	2	4

(iii) Female choice

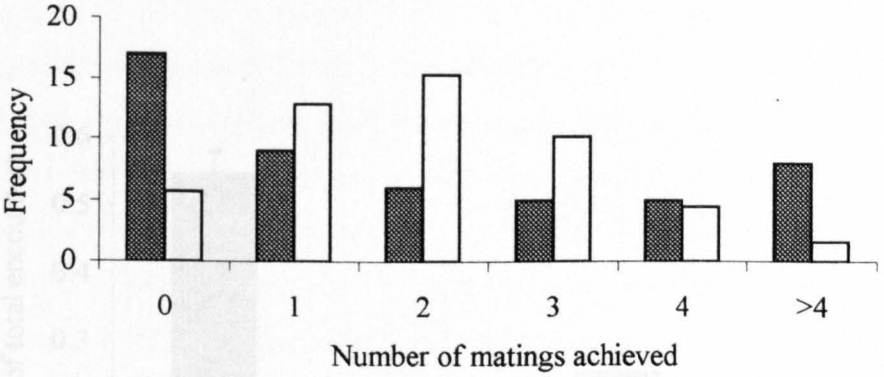
Variance in male mating success

Male mating success was consistently skewed across trials, such that a few males achieved a large proportion of the available matings and the remaining males achieved few or no matings (Figures 3.7a-c). In all trials, the observed frequency distributions of matings differed significantly from the random expectations generated by computer simulations. The frequency distribution of matings did not vary with female feeding status (comparison of mating frequencies in 5 male trials using fed and unfed females: $F_{1,11} = 0.02$, NS). A male's mating success was also not dependent on the colour of fluorescent dye used to mark him (comparison of male mating success with respect to dye colour: 5 males, fed females: $F_{4,49} = 1.34$; 5 males, unfed females: $F_{4,49} = 2.04$; 3 males, $F_{2,27} = 1.81$, all NS).

(a) Unfed females, 5 males: $\chi_5^2 = 21.33$, $n = 10$ trials, $P < 0.001$



(b) Fed females, 5 males: $\chi_5^2 = 26.09$, $n = 10$ trials, $P < 0.001$



(c) Fed females, 3 males: $\chi_4^2 = 5.93$, $n = 10$ trials, $P < 0.05$

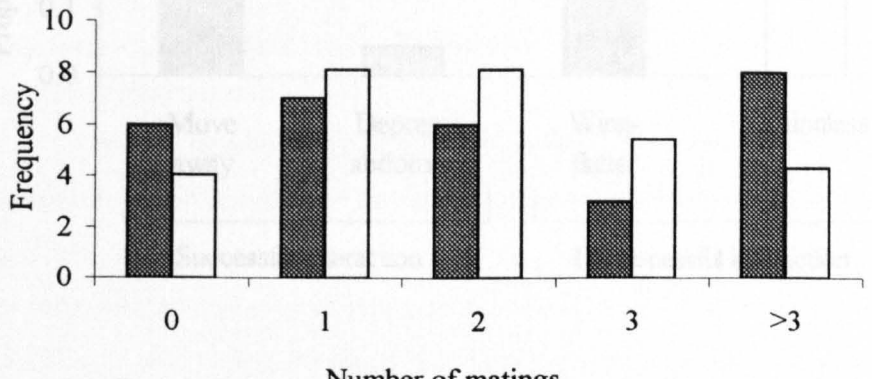


Figure 3.7. A comparison of the observed and expected distributions of matings across three sorts of trials (a) Unfed females, 5 males; (b) Fed females, 5 males; (c) Fed females, 3 males. ■ Observed □ Expected

Role of female choice

In all 30 trials run to examine the role of female choice, all females mated. Prior to copulating, females approached a mean (± s.e.) of 3.30 ± 0.04 of the 10 males available. The majority of encounters between males and females resulted in the female walking or flying away prior to physical contact **(Figure 3.8).** Occasionally a female terminated an attempted copulation by depressing her abdomen to the ground, thereby forcing the abdomen of the male away from her own. All remaining encounters with males resulted in successful copulations and involved either a female wing-fluttering at the approaching male, or remaining motionless while a male copulated. After copulating, males usually moved away from a female and began wing-fluttering again.

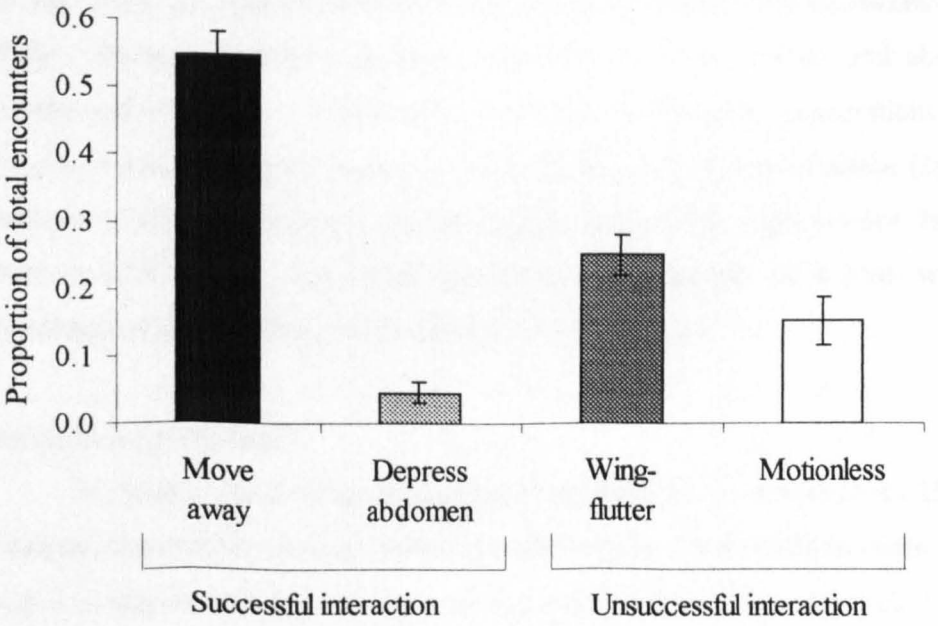


Figure 3.8. Female behaviour on encountering a male. Data were obtained from 30 females with a combined total of 54 interactions with males.

Discussion

The results in these experiments support the idea that the sibling species of *L. longipalpis* found on Marajó is a lek-breeding insect *sensu strictu* (Bradbury 1981).

Male aggregation and agonistic behaviour

In their natural environment, males formed stable spatial aggregations on or near hosts in chicken sheds (see also Dye *et al.* 1991). On a host males performed wing-fluttering displays, and engaged in occasional fights with other males. In the laboratory, males tended to form stable aggregations in the lower half of the cage irrespective of host presence, but did not aggregate on the host when present. Agonistic behaviour observed in the laboratory was comparable to that seen in the chicken sheds and typically involved males wing-fluttering at other approaching males. Within laboratory-aggregations, males were active in the presence and absence of females and occasionally fought with one another. While these observations of male agonistic behaviour in the laboratory are comparable with previous findings (Jarvis and Rutledge 1992), my data on the distribution of males within cages are not. Jarvis and Rutledge (1992) found that males aggregated predominantly on a host, whereas I found aggregation position was unaffected by host presence.

Importance of resources

In chicken sheds, males aggregated predominantly on or near hosts. However, because I observed no matings during my observations, I am unable to assess whether access to resources determines the mating success of free ranging females. Two lines of laboratory evidence suggest this may not be the case. First, males aggregated in the lower region of the cage irrespective of host presence, and did not aggregate on the host when it was present. Second, the presence of a host seemed to have no effect on the mating behaviour of caged females: they mated equally often in the presence and absence of a host and indeed only one female mated on a host. That copulation occurred prior to the female feeding and the female then moved to a separate location

to feed. Further investigation is required to assess how far these laboratory findings reflect events in the field.

Female choice

The mating success of caged male aggregations was consistently skewed, such that a few males obtained the majority of the available matings. This is typical of many lekking species (Bradbury *et al.* 1985, Wiley 1991, Höglund and Alatalo 1995). Although it is not possible to distinguish completely the roles of male-male interactions and female choice, the evidence presented suggests that female choice is at least partially responsible for determining a male's mating success. Females were observed to sample and reject several males prior to a successful copulation, and were able to reject males even during a copulation by rapid depression of their abdomens. In addition, a male's ability to win fights is not correlated with his mating success (Chapter 7), suggesting that the results of male-male interactions do not determine female access to potential mates.

Differences between the two studies

Several of my results differed from these of Jarvis and Rutledge (1992). The present experiments showed that males did not aggregate on an available host, and that virgin females mated readily in the laboratory and within a short period after exposure to males. In contrast, Jarvis and Rutledge (1992) found that males aggregated mostly on a host and that females were reluctant to mate: only 8% mated within one hour of introduction to males and only 55% had mated after 24hr.

There is a methodological reason which may explain the difference in the proportions of mated females that were observed. In my experience, females released more than 12hr after emergence may not be virgins. Since mated females are reluctant to remate (Chapter 2) and Jarvis and Rutledge released flies 24hr after eclosion, it is probable that some of their females were not virgin at the start of the trial. The number of females willing to accept a mating would therefore be reduced. This does not,

however, explain why males should be less willing to aggregate on a host in the present experiment. There are at least two probable causes for this difference. The differences may be caused by age-related differences in male behaviour. I used 4-6d old males throughout my trials, whereas Jarvis and Rutledge (1992) used males differing in age and their aggregations on hosts were mostly made up of older males. An alternative explanation is that the *L. longipalpis* populations used in the two studies might be different sibling species with distinct mating systems. In addition to the behavioural differences reported here, the pheromones distributed by displaying males differ chemically across the two populations (J.G.C. Hamilton pers. comm.). Laboratory crosses and direct comparison of caged flies of both populations would enable this hypothesis to be tested further.

Conclusion

In conclusion, the experiments outlined here confirm that the mating aggregations of *L. longipalpis* from Marajó Island constitute leks (Bradbury 1981, 1985). The observations that males aggregate readily in the laboratory and that females mate freely from within these aggregations even in the absence of a host all mean that *L. longipalpis* is an ideal species to use in studies of sexual selection. Moreover, the fact that male mating success was highly skewed suggests that even after 20 generations as a laboratory-bred colony, the population has maintained some degree of genetic variability.

Chapter 4

Evaluating models for the evolution of lekking: a field and laboratory study.

Summary

1. Field and laboratory experiments were carried out to investigate possible mechanisms promoting the evolution of the lek-mating system found in *Lutzomyia longipalpis*.
2. In the field, the distribution of resources used by *L. longipalpis* was manipulated by using cages containing varying numbers of chickens, and the distribution of males and females then investigated. Measures of male morphology were taken to assess the relationship between male size and lek size.
3. The number of males attracted to a cage (lek size) increased with the number of hosts present. On average males on larger leks had longer abdomens, but the variance in male size was constant across leks. The number of females was positively correlated with the number of males present, but the average number of females per male decreased with increasing lek size.
4. In the laboratory, single females were introduced to leks of different sizes, and the female latency to mating was compared across leks. Females exposed to larger leks encountered and copulated with males sooner than those exposed to smaller aggregations, but the length of time a pair spent courting did not vary with lek size.
5. It is suggested that the study provides limited support for both hotspots and a female preference for large leks as possible factors promoting the evolution of lekking in *L. longipalpis*: the distribution of males is influenced by the distribution of resources while the number of females is positively correlated with lek size and unrelated to resource distribution. I reject the black-hole model as a potential explanation for lekking in this species because, contrary to an explicit prediction of the model, lek size had a direct effect on a female's latency to mate.

Introduction

Any model for the evolution of lekking must account for the spatial distribution of tightly clustered groups of males. The aim of this chapter is to attempt to discriminate between several of the current models by experimentally exploring the relationships between the distributions of resources, males and females in lekking sandflies. Below is a brief review of the most widely accepted models proposed for the evolution of lekking and a summary of the predictions they make about these relationships (summarised in Table 4.1). For a more detailed and mathematical description of the models the reader is referred to Höglund and Alatalo (1995) and Sutherland (1996).

Hotspot models

Simple hotspot models (Bradbury *et al.* 1986, McNaughton 1988, Apollonio 1989, Gosling and Petrie 1990, Gibson 1996a) are based on the ideal free distribution of equal competitors (Fretwell and Lucas 1970, Sutherland 1983). They suggest that competitively equal males distribute themselves in order to maximise their access to females, whose distribution is in turn determined by the clustering of non-defensible resources. The models predict that because female distribution is influenced solely by that of resources, the extent of male clumping increases as resources become more aggregated. Hotspot models predict a positive correlation between the number of males on leks (lek size) and number of females, but because males distribute themselves in an ideal free manner, average male mating success and the average number of females per male are both independent of lek size.

Hotshot models

Hotspot models were challenged by Beehler and Foster (1988) and Höglund and Robertson (1990), who proposed instead that male clumping arises not because of male clustering around resources, but because certain males ("hotshots") enjoy disproportionate mating success, either due to their superiority in male-male competitive interactions or because females find them particularly

attractive. In the formal hotshot model and attractiveness models (Beehler and Foster 1988, Höglund and Robertson 1990) and the earlier kleptoparasitism models (Arak 1983, 1988), lower quality males cluster around hotshots in an attempt to increase their reproductive success by intercepting females that are attracted to the more successful males. These models make no explicit predictions about the relationship between female attendance and lek size, but if large leks form around particularly attractive hotshots, the overall number of females visiting a lek should increase with lek size (Beehler and Foster 1988). By the same argument, these models infer that as lek size increases variance in male quality should increase. Lastly, in contrast to the hotspot model, hotshot models specifically predict that the distribution of males is unrelated to the distribution of resources.

In their simplest form, both hotspot and hotshot models are faced with fundamental problems. On the one hand, neither model is able to account for the degree of clumping found in many lekking species (Bradbury 1981, Sutherland 1996). On the other, the hotshot mechanism does not explain why parasitised males remain with lower quality males, rather than moving away and taking their females with them (Höglund and Alatalo 1995). Two subsequent models - the ideal free distribution of unequal competitors, (Sutherland and Parker 1985, Sutherland *et al.* 1988) and the skew model (Widemo and Owens 1995) - were proposed to offer increasingly sophisticated descriptions of reality.

Ideal free distribution of unequal competitors (IFDUC)

In this model (Sutherland and Parker 1985, Sutherland *et al.* 1988; see also Sutherland 1996), males differing in their ability to attract or compete for mates distribute themselves in an ideal free manner across sites which differ in female arrival rates. Explicit modelling of male settlement patterns reveals that high quality males should settle at sites with the highest female arrival rates. Inferior males achieve consistently lower access to females, but compete equally well at sites with both low and intermediate female arrival rates. As a consequence of the distribution of males, there should be a positive relationship between lek size and

both overall female arrival rate and overall male mating success. In addition, average male quality is predicted to increase with lek size (Sutherland 1996).

There are two main problems with this model. First, despite combining a hotspot process with differences in male competitive ability, the model still cannot fully account for the extent of male clustering which has been observed empirically, requiring some additional process to be invoked (Sutherland 1996). Second, the model assumes that skew in male mating success is independent of lek size. This may not hold true if, for example, successful males lose mating opportunities while defending their territories against competitors - this would lead to a decrease in mating skew with increasing lek size. Both these problems are addressed in the skew model (Widemo and Owens 1995).

Skew model

Widemo and Owens (1995) incorporated elements of both the hotspot and hotshot models, allowing males differing in rank to distribute at sites pre-determined by female spatial preference. An important feature incorporated into the skew model is the empirically observed negative relationship between male mating skew and lek size (Alatalo *et al.* 1992, Höglund *et al.* 1993). In their model Widemo and Owens (1995) showed that the interactions between lek size and overall copulation rate, and lek size and male mating skew may result in a conflict of interest between males of differing rank with respect to optimal lek size. In comparison with the previous model, they argue that this conflict of interest between males promotes male clustering as low ranking males join high ranking males at those sites where females are most commonly encountered. Low ranking males are more likely to obtain a mating on a larger lek where mating skew and the ability of top males to monopolise matings is reduced. The model predicts that males aggregate at hotspots and thus that male distribution is influenced by resource clumping. In addition it suggests that the total number of females increases with lek size on smaller leks, but at the largest leks this positive relationship may flatten out. This may come about as a result of high levels of intense male-male interactions which could deter females (Widemo pers. comm.).

Finally, if the model's predictions hold, then it follows that variance in male quality increases with lek size as a result of low ranking males clustering around hotshots.

While the preceding models hinge on females aggregating at particular sites or with particular males, two of the remaining groups of models suggest female aggregation is a result of male clustering, because clumped males are in some way better at attracting or retaining females.

Female preference models

Female preference models suggest that females may actively prefer to mate in larger aggregations either because they facilitate comparison of several males at low search cost (Alexander 1975, Wrangham 1980, Bradbury 1981), or because they offer safe sites from predators (Lack 1968, Wiley 1973, Wittenberger 1978). Strict application of the models generates the prediction that all females should go to the largest lek in their home range (Alexander 1975, Bradbury 1981). This extreme interpretation is however not supported by empirical data as smaller leks also attract limited numbers of females (Kruijt *et al.* 1972, Alatalo *et al.* 1992, Lank and Smith 1992). In contrast to hotspot, IFDUC and skew models, female preference models do not predict a correlation between the distribution of males and females and that of resources.

Black hole model

According to the black hole model, leks may arise as a consequence of female movement between territories. If females typically move between neighbouring territories several times prior to mating, they may tend to be retained in territories that are clustered (Stillman *et al.* 1996). While the models do not specify what stimulates female movement, Stillman *et al.* (1996) suggest that sampling of mates could be a potential explanation, although male harassment or predation may be a more common cause (Clutton-Brock *et al.* 1992, Stillman *et al.* 1993). The black hole model makes no obvious predictions about the relationships between female distribution or male phenotype and lek size. However, it suggests that there is no need to invoke non-random mating behaviour by females and

predicts that the time from female arrival at a lek to mating should be independent of lek size.

Kin selection model

Finally, in the most recently proposed model, Kokko and Lindström (1996b) invoke kin selection as a mechanism promoting the formation of leks. In their model, they consider the direct and indirect fitness consequences to males of displaying on leks. They show that less successful males benefit from displaying on the same lek as their fathers as they gain indirect fitness benefits which override the loss of direct mating opportunities. This mechanism thus promotes clustering of younger males around their older, more experienced, fathers. The model predicts an increase in the number of females on larger leks, but makes no predictions about the distribution of males and females with respect to resources. While variance between males is not explicitly considered, if younger, lower-ranked males cluster around their higher ranking fathers it follows that variance between males should increase with lek size. While included in this review for completeness, I will not attempt to assess the predictions of this model in this chapter as they do not hold for species, such as *Lutzomyia longipalpis*, that have discrete generations.

This study

Preliminary field results in *L. longipalpis* indicate that lek size is in part determined by the numbers of hosts present at a site and that the numbers of females attracted to a site increases with increasing number of males (Kelly *et al.* 1996). The present study was divided into two parts. First, in the field, the distribution of potential resources was manipulated using cages containing varying numbers of chickens. Males and females were allowed to distribute freely across all available sites. Second, lek size was manipulated in laboratory cages and female latency to copulation measured. Data from these two experiments were used to explore the following relationships (see Table 4.1): (a) distribution of males versus clustering of resources; (b) male phenotype versus lek size; (c) distribution of females versus lek size; (d) female latency to mate versus lek size.

Table 4.1. Review of a number of the predictions of current models for the evolution of lekking.

Relationship / Model	Distribution of males versus clustering of resources	Male phenotype versus lek size	Distribution of females versus lek size	Female latency to mate versus lek size
Hotspot models (Bradbury *et al.* 1986, McNaughton 1988, Apollonio 1989, Gosling and Petrie 1990, Gibson 1996a)	Male distribution dependent on available resources.	No difference in male quality.	Number of females increases with lek size. Average number per male constant across leks.	No prediction.
Hotshot models (Arak 1983, 1988, Beehler and Foster 1988, Höglund and Robertson 1990)	Male distribution independent of available resources.	Variance in male quality increases with lek size.	No explicit prediction, but likely that number of females increases with lek size.	No prediction.
Ideal free distribution of unequal competitors (Sutherland and Parker 1985, Sutherland *et al.* 1988)	Male distribution dependent on available resources.	Mean male quality increases with lek size.	Number of females increases with lek size. Average number per male increases with lek size.	No prediction.
Skew model (Widemo and Owens 1995)	Male distribution dependent on available resources.	Variance in male quality increases with lek size.	Total number increases with lek size on smaller leks, but positive relationship flattens out on the largest leks.	No prediction.

Table 4.1. (cont.)

Relationship / Model	Distribution of males versus clustering of resources	Male phenotype versus lek size	Distribution of females versus lek size	Female latency to mate versus lek size
Female preference models (Lack 1968, Wittenberger 1978, Bradbury 1981)	Male distribution independent of available resources.	No prediction.	Number of females increases with lek size.	No prediction.
Black hole model (Clutton-Brock *et al.* 1992, Stillman *et al.* 1993)	Male distribution independent of available resources.	No prediction.	No prediction.	No difference in latency to mate as lek size increases.
Kin selection model (Kokko and Lindström 1996b)	No prediction.	Variance in male quality increases with lek size.	Number of females increases with lek size.	No prediction.

Methods

(i) Field manipulation of resources

The aim of this experiment was to manipulate the distribution of food resources used by female sandflies and to examine the consequences for the distribution of both lekking males and females. Resource dispersion was manipulated by placing one to six chickens in each of seven experimental cages arranged in two sites in the village of Pingo d'Água, Marajó, Brazil (Chapter 2). The sites were approximately 200m apart, and had both yielded large numbers of

flies in the past two years (R. Quinnell pers. comm.) and immediately prior to this experiment (pers. obs.). Note that, while in the same village, they were also at different locations to those used in Chapter 3. Cages were constructed out of 5cm wooden slats, and housed the chickens normally resident in the shed closest to the chosen site. Large cages (80x80x40cm) housed three to six chickens; medium cages (60x60x40cm), two to three chickens and small cages (20x40x20cm) one chicken. Cages were placed outside resident chicken sheds, in a triangle (three cages, site 1) or square (four cages, site 2) arrangement, approximately 5m apart from their nearest neighbour. Cages were rotated nightly within a site to minimise positional effects. It is assumed that the males caught in an individual cage represent a single lek. This assumption is supported by field observations of lek formation (Chapter 3) and also by the fact that chickens tend to huddle into a group when placed in a confined cage (pers. obs.).

Sampling was conducted for two to four day periods, at monthly intervals from 5 August to 28 October, 1995. If it rained during an evening, the capture was aborted for that night. Each night, sandfly proof bags were placed under each cage, and all chickens normally resident at a site were captured as they returned to roost and secured in the experimental cages. The cages were then left undisturbed until 21:00hr, when the bags were carefully rolled over the cage and then tightly tied, to avoid loss of flies. All bags were tied within a 10min period. After the flies were securely enclosed, all cages were placed individually in a large sandfly proof enclosure (100x100x150cm). The flies from a single lek were released into the enclosure and aspirated immediately. After aspiration of flies from the walls of the enclosure, the chickens were checked to ensure that any remaining flies were removed. All flies were then transferred to 80% alcohol.

Within 12hr of capture, flies were sexed and counted. To assess male phenotype in relation to lek size, up to 10 intact males were randomly selected from each sample and morphological measurements of length (left and right wing, abdomen, thorax) were taken using a digitised computer system attached to a binocular microscope. Males showing capture damage were discarded. Wing length

was defined as the distance from the apex at vein R5 to the point where R5 meets R2 and M2 (after Kelly *et al.* 1996). A total of 20 males were randomly selected and remeasured to assess repeatability of the technique used. Morphological measurements were repeatable (Intraclass correlations; Zar 1984: left wing length: $r_I = 0.91$; right wing length: $r_I = 0.87$; abdomen length: $r_I = 0.86$; thorax length: $r_I = 0.91$; $n = 20$, $P < 0.01$ respectively).

The numbers of males and females caught in cages and the numbers of chickens were log-transformed to normalise the data set. Factors affecting the distribution of males were analysed using multiple ANCOVAs in GLIM 4.0. Log(numbers of males + 1) was set as the dependent variable, log number of chickens was entered as a continuous variable. Date and site of capture were entered as factors with nine and two levels respectively. Females were not entered into this model as they arrive at a site after males (Kelly *et al.* 1996) and are thus not likely to directly influence the distribution of males. Factors affecting the distribution of females were analysed in a similar manner with log (numbers of females + 1) as the dependent variable, number of males and number of chickens as continuous variables, and date and site of collection as factors with 9 and 2 levels respectively. In both models the significance of each parameter was assessed by stepwise deletion as described in Chapter 2. The effect of cage position on the total numbers of flies caught was analysed for each site individually using ANOVAs.

Mean male morphology and variance in male morphology were compared across leks and sites using multiple ANCOVAs in GLIM 4.0. The measured character was set as the dependent variable with lek size included as a continuous variable and site of collection included as a factor with two levels. The significance of the parameters was assessed by step-wise deletion (Chapter 2). After correction for five multiple comparisons (Chapter 2) the 95% significance level is $P < 0.01$.

(ii) Female latency to mate versus lek size

To explore the effect of lek size on female latency to mate, females were exposed to caged leks of differing sizes, and their time to copulation monitored. All flies used during the experiment were 4-6d old virgins, separated by sex less than 12hr after adult emergence. Aggregations of 1, 2, 4, 8, 16, 32, 64, 128 males were introduced into net cages. After a ten minute equilibrium period, a single 4-5d old female (fed 24hr earlier) was introduced into each cage. The range of female:male ratios in the cages mirrored the range of values observed in the field (Table 4.2). I then measured the time between introduction of a female and termination of copulation, divided into a number of stages:

(a) *Initial encounter*: time from introduction to when the female is first within 2cm of a male.
(b) *Encounter - courtship*: time between initial encounter and the onset of courtship, defined as the point at which a female flutters her wings at an approaching, wing-fluttering male.
(c) *Courtship*: time between the start of courtship to the start of copulation. A pair were assumed to have commenced copulating when the male and female were seen to be joined at the abdomen.
(d) *Copulation*: time taken from the initial joining of the male and female abdomens to their separation.
(e) *Total time*: the sum of (a) - (d).

Following copulation, the mating pair was removed and for leks ≤ 8 males, all males were replaced. For larger leks (>8 males), only the copulating male was replaced. A total of 10 females was introduced per aggregation size and each female was used only once. By introducing individual females into the cages and removing mated males after copulation, the potentially confounding effects of female copying or of changes in male behaviour as a result of copulation were thus eliminated (Gibson and Höglund 1992, McComb and Clutton-Brock 1994, Kirkpatrick and Dugatkin 1994).

Laboratory data were log-transformed and where possible analysed using least squares regression. The variance:mean ratio of copulation lengths was approximately constant after transformation, thus the relationship between the length of copulation and lek size was analysed in GLIM 4.0 assuming a Gamma error distribution (after Crawley 1993). Results were corrected for five multiple comparisons as above.

Results

(i) Field manipulation of resources

Flies were caught on eight nights at site 1 and nine nights at site 2, for a total of 60 (= [8x3] + [9x4]) cage nights. On each night, males and females generally distributed themselves across all available cages within a site, although numbers of females caught were consistently lower than numbers of males. There was a large variation in both lek size and sex ratio of captures (Table 4.2).

Table 4.2. Sample sizes for captures made in each site at Pingo d'Água.

Flies at site	Site 1		Site 2	
	Males	Females	Males	Females
Geometric mean numbers of flies	30.4	1.6	8.5	0.6
Range of numbers of flies	0-562	0-14	0-11	0-5
Minimum ratio of females to males	1:8		1:2	
Maximum ratio of females to males	1:100		1:67	
Mean ratio of females to males	1:25		1:27	

Distribution of males versus clustering of resources

The number of males found in each cage increased with the number of chickens present ($F_{1,58} = 19.68$, $P < 0.01$, Figure 4.1), and varied consistently between sites ($F_{1,58} = 19.00$, $P < 0.01$). There was no evidence to suggest between night variation in the numbers of males captured ($F_{8,57} = 0.19$, NS). Interactions terms were consistently non-significant and are thus not reported. The number of males found in each cage was independent of the position of a cage within a site (site 1: $F_{2,23} = 2.12$; site 2: $F_{3,35} = 0.44$, NS respectively).

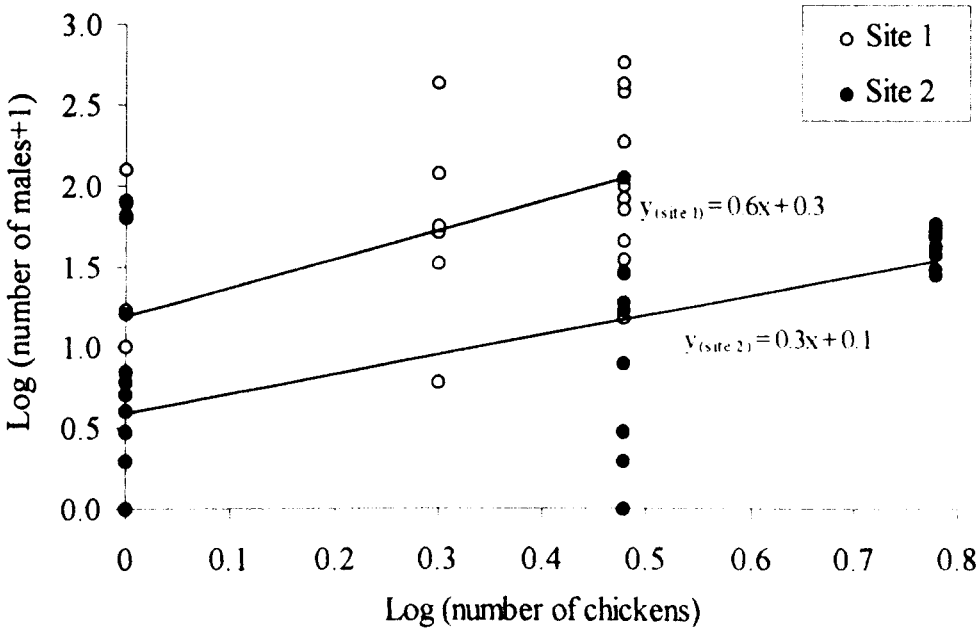


Figure 4.1. Relationship between log(number of males+1) and log(number of chickens) across sites 1 and 2.

Male phenotype versus lek size

The data indicate a significant relationship between one aspect of male size and lek size: males attending larger leks had significantly longer abdomens than males present on smaller leks (Table 4.3, Figure 4.2). Abdomen length was

uncorrelated with other morphological measurements (Table 4.4). Wing and thorax lengths were intercorrelated (Table 4.4), although were unrelated to lek size (Table 4.3). The apparent difference in thorax length between sites was non-significant after correction for multiple comparisons. No other measures of mean male size varied significantly between sites (Table 4.3). Furthermore, there was no evidence that variance in any aspect of male morphology differed between sites or across leks of different sizes (Table 4.5).

Table 4.3. Results of multiple ANCOVAs investigating the relationship between mean male size and lek size across sites. Uncorrected significance values are given; NS = not significant.

Character measured	Lek size (n = 45 males)	Site (n = 45 males)
Left wing length	F = 1.49, NS	F = 0.04, NS
Right wing length	F = 0.75, NS	F = 0.01, NS
Abdomen length	F = 8.60, P < 0.01	F = 0.03, NS
Thorax length	F = 1.54, NS	F = 5.85, P < 0.05

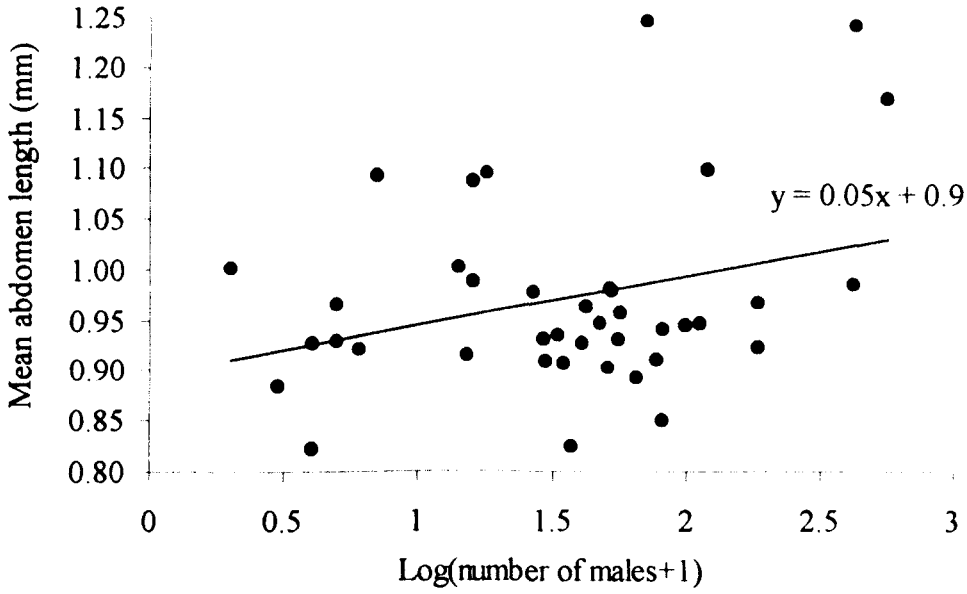


Figure 4.2. Relationship between the mean abdomen length of males and lek size.

Table 4.4. Correlation coefficients between morphometric measures of 45 adult males. Uncorrected significance values are given by: NS = not significant, *** = $P < 0.001$. After correction for multiple comparisons the 95% significance level is $P < 0.01$.

	Left wing	Right wing	Abdomen
Right wing	0.88 ***		
Abdomen	0.03 NS	-0.03 NS	
Thorax	0.70 ***	0.63 ***	0.13 NS

Table 4.5. Results of multiple ANCOVAs investigating the relationship between variance in male size and lek size across sites. Notation as in Table 4.3.

Character measured	Lek size (n = 45 males)	Site (n = 45 males)
Left wing length	F = 0.04, NS	F = 0.11, NS
Right wing length	F =0.07, NS	F = 0.36, NS
Abdomen length	F =0.17, NS	F = 0.51, NS
Thorax length	F =0.20, NS	F = 3.04, NS

Distribution of females versus lek size

The number of female flies found in each cage increased with the number of males present ($F_{1,59}$ = 32.19, $P < 0.01$; Figure 4.3). The slope of the log-log plot was significantly less than unity (slope [±s.e.] = 0.27 [±0.02], t = 36.94, n = 60, $P < 0.001$), indicating that the ratio of females to males decreases with increasing male number. There was no evidence to suggest that the number of females varied across nights, between sites or with numbers of chickens present in each cage (effect of night: $F_{8,59}$ = 1.44; site: $F_{1,59}$ = 1.44; number of chickens: $F_{1,58}$ = 0.18; all NS). Interaction terms were consistently non significant and are thus not reported. The numbers of females found in each cage was independent of the position of the cage within a site (site 1: $F_{2,15}$ = 0.84, site 2: $F_{3,26}$ = 0.28, all NS).

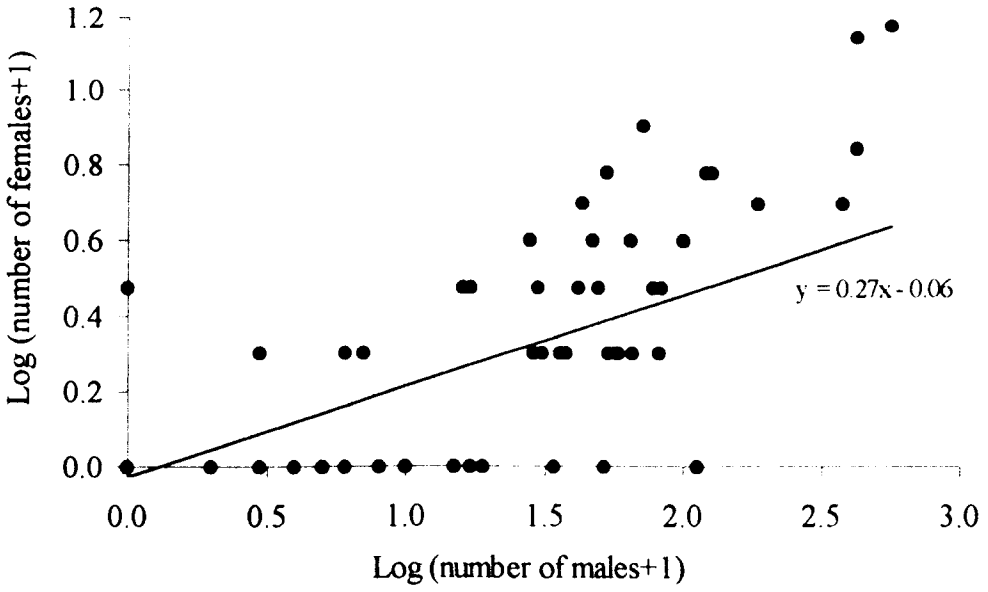


Figure 4.3. Relationship between the numbers of females and males across all 60 cage nights. The equation of the line was derived from the minimum adequate model, described in the text.

(ii) Female latency to mate versus lek size.

Although all females introduced into the cages mated within ten minutes, the length of time that elapsed before mating varied strongly with lek size. Females exposed to leks with fewer males took longer to encounter a male and also spent longer between their initial encounter and courtship than females exposed to larger aggregations (regression analyses: time to initial encounter: $r = 0.58$, $n = 76$; encounter - courtship: $r = 0.39$, $n = 68$, $P < 0.001$ respectively; Figures 4.4a-b; differences in n are due to missed observations). Courtship duration did not vary with number of males ($r = 0.04$, $n = 65$, NS; Figure 4.4c), but the length of copulation increased with lek size ($r = 0.41$, $n = 80$, $P < 0.001$, Figure 4.4d). Overall, females spent significantly less time from introduction to the end of copulation when exposed to larger leks (total time: $r = 0.38$, $n = 80$, $P < 0.001$, Figure 4.4e).

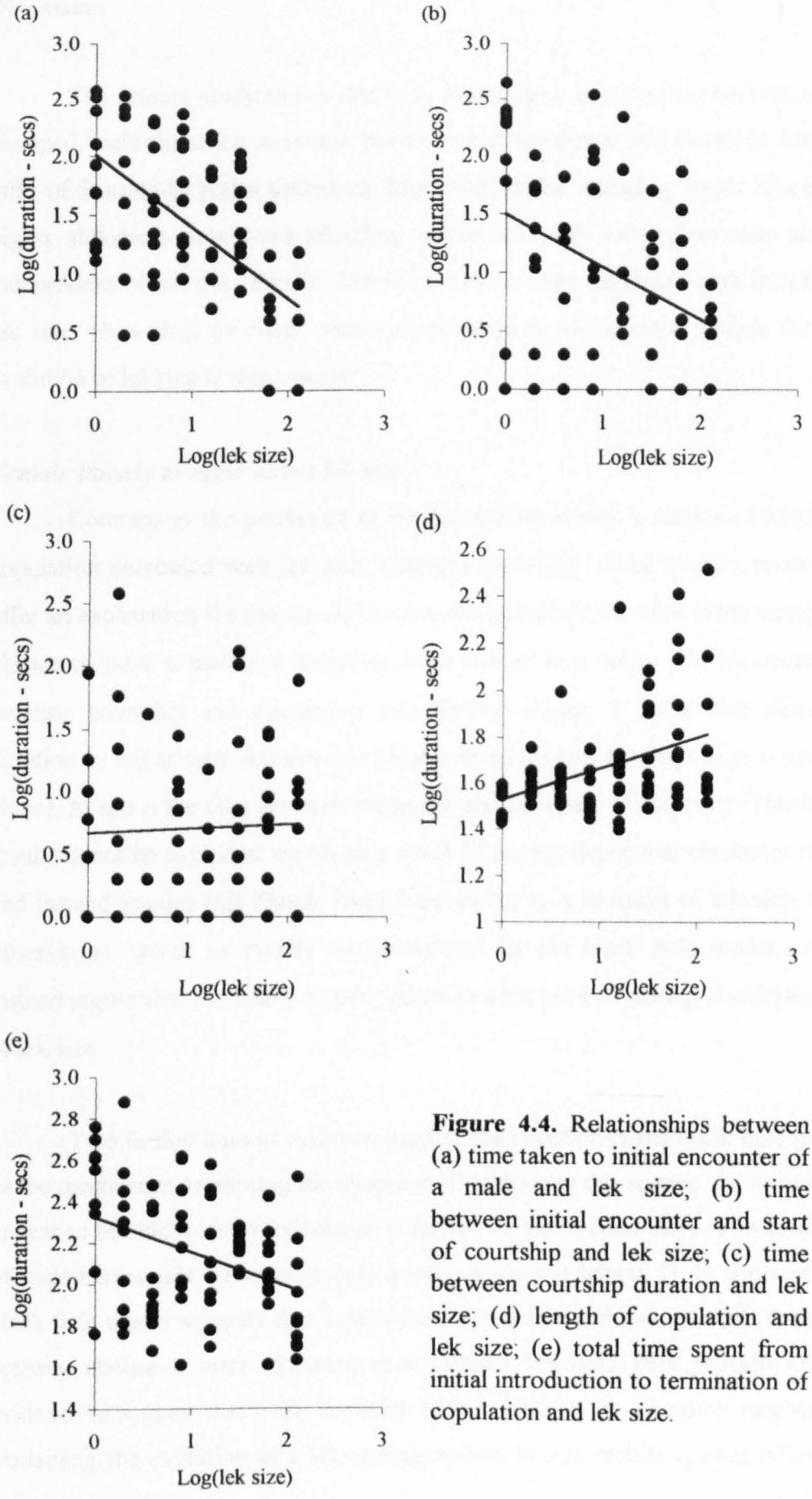


Figure 4.4. Relationships between (a) time taken to initial encounter of a male and lek size; (b) time between initial encounter and start of courtship and lek size; (c) time between courtship duration and lek size; (d) length of copulation and lek size; (e) total time spent from initial introduction to termination of copulation and lek size.

Discussion

The present study shows that in *L. longipalpis*, as resources become more clumped, male clustering increases, the number of females at leks increases, but the ratio of females to males decreases. Moreover, males attending larger leks have bigger abdomens than males attending smaller leks, but variance in male size is independent of lek size. Finally, female latency to mate decreases with increasing lek size. How then do these observations relate to the current models for the evolution of lekking in this species?

Female latency to mate versus lek size

Contrary to the prediction of the black hole model, a female's latency to copulation decreased with lek size. Changes in density alone could conceivably offer an explanation for this result, because females might be more likely simply by chance to meet a male and therefore mate sooner in a larger lek. However, by dividing courtship and copulation into distinct stages, I found that although duration to initial male encounter is shorter in larger leks (perhaps due to density alone), so too is the time between encounter and the onset of courtship. This latter result cannot be explained simply as a result of density-dependent encounter rates, and instead implies that female receptivity varies as a function of lek size. This observation cannot be readily accommodated by the black hole model, which instead argues that the time a female spends in a lek prior to mating is independent of lek size.

Two further lines of evidence support the rejection of the black hole model as the mechanism promoting the evolution of lekking in this species. First, females appear to be able to exert free choice (Chapter 3), and second there is no evidence of male harassment influencing male mating success (Chapter 3). In contrast, the black hole model suggests that harassment is prevalent and that a female does not actively choose a mate (Stillman *et al.* 1996). My data thus provide further evidence to suggest that while the black hole model may be a possible mechanism explaining the evolution of a lek mating system in less mobile species (Clutton-

Brock *et. al.* 1992), it perhaps does not provide an adequate model for highly mobile organisms such as *L. longipalpis* (see Höglund and Alatalo 1995, Sutherland 1996).

Distribution of males versus clustering of resources

If numbers of males are positively correlated with the clustering of resources, it implies that males are initially distributing themselves with respect to hotspots. Several studies have explicitly investigated the predictions of the hotspot model as a mechanism promoting the distribution of males (Bradbury *et al.* 1989, Balmford *et al.* 1993, Westcott 1994, Wickman *et al.* 1995). This study, however, provides the first evidence to my knowledge that males respond positively to manipulation of potential hotspots in the field. The fact that males aggregate at resources required by females does not refute the basic definition of lek-breeding, because variation in male mating success within leks is not determined by their differential access to resources (Bradbury 1985; see Chapter 3). The causal link between clumping of resources and of males supports the predictions of simple hotspot models, the IFDUC and the skew model.

Male phenotype versus lek size

The skew model and hotshot models all predict an increase in the variance between males with lek size. In contrast, the IFDUC model proposes that the highest quality males occupy the largest leks. Here I found variance in male morphology to be consistent across leks. However, males attending large leks had longer abdomens than males attending small leks. A limitation in the interpretation of these results is that the relationship between male size and quality is not known. This is further constrained by the lack of data on numbers of copulations obtained by individual males. If differences in size are related to a male's competitive ability (see below for discussion), these results refute the predictions of the skew and the simple hotshot models and highlight the IFDUC as a possible model explaining the evolution of lekking in this species. However, the IFDUC model may be inadequate as it predicts even less clustering of males than either the hotspot or the skew models (Widemo 1995). Without measures of the mating success of

individual males or knowledge of the relationship between male size and competitive ability, it is not possible to distinguish between these hypotheses further.

Distribution of females versus lek size

While males distribute themselves according to a hotspot-type process, females appear to be more sensitive to the distribution of males than that of resources. The fact that the distribution of females is influenced more strongly by males than hosts, coupled with the knowledge that female attraction to a site is mediated by male pheromones (Ward *et al.* 1988, Kelly and Dye 1997), suggests a role for female preference models in the evolution of this lek system. If the female preference models hold then males would benefit by clustering, because females preferentially visit, or are more attracted to the largest leks.

However, this does not explain why, despite the increase in number of females with lek size, the ratio of females to males decreased on larger leks. Limited evidence from other species provides mixed support for the prediction of hotspot and IFDUC models of an increase in per capita male mating success with increasing lek size. For example, in the Uganda kob, *Kobus kob thomasi*, Balmford *et al.* (1992) found that average male mating success increased with lek size, but a more recent study found that, despite a close correlation between the numbers of females and the numbers of males present, the average number of females per male did not increase with lek size (Deutsch 1994). In sage grouse, *Centrocercus urophasianus*, leks of up 25-50 males are found to attract increasing numbers of visits from females, but as lek size increases beyond this the number of females visiting levels off (Bradbury *et al.* 1989). In insects, empirical evidence is again inconsistent. In the sex-role reversed dance fly *Empis borealis*, large leks attract the highest number of male visits per female (Svensson and Petersson 1992) and in *Drosophila conformis*, male mating success increases with lek size (Shelly 1990). In chironomid midges, *Chironomus plumosus*, the pattern is somewhat more complicated: males in the smallest and the very largest swarms achieve the highest

mating success, whereas males in average-sized swarms attract fewer females on a per capita basis (Neems *et. al.* 1992).

In *L. longipalpis* the signalling system used for host location and aggregation may provide a mechanistic explanation for the observed decrease in the average numbers of females per male in larger leks. Males and females are attracted to a host by pheromones thought to be distributed by the wing-fluttering behaviour of resident males (see Chapter 2). Signalling theory predicts a constraint on the signal output, such that the total amount of signal produced does not increase linearly with the numbers of signallers present (Bradbury 1981 and references therein). If female attraction to a site is mediated primarily by pheromones, the average numbers of females attracted per male is thus likely to decrease with increasing lek size. This relationship would be further amplified if males were disproportionately more attracted to pheromones than females. However, if a male's reproductive success is related to his size, why should large males remain on larger leks despite the apparent reduction in access to females? Three possible explanations are offered to explain this observation.

First, as has been found in other species of insects, it is possible that in *L. longipalpis* small males have a superior mating advantage. For example, in midges, *C. plumosus*, small males have a mating advantage over larger males by being more agile, and they congregate in the smallest swarms where mating success if highest (McLachlan and Allen 1987, Neems *et al.* 1990, Neems *et al.* 1992). Likewise, in *Drosophila melanogaster* small males achieve higher mating success than larger males (Steele and Partridge 1988). While there is no evidence in this study of a relationship between male mating success and male size (see Chapter 6), it is unlikely that small size is advantageous in *L. longipalpis*, where males jostle and fight to retain their position in a lek (Jarvis and Rutledge 1992) and attract females using pheromone signalling (Ward *et al.* 1988). Potential effects of body size in the field could have been explored in the present field experiment by assessing the mating success or survival of males after capture.

A second possibility is that lek selection by males is limited by perceptual constraints (Abrahams 1986, Neems *et al.* 1992). In *L. longipalpis*, the sizes of leks stabilise prior to mass immigration of females (Kelly and Dye 1997), so that males assess the suitability of leks by host presence and the number of males already present (promoted by host kairomones and male pheromones), rather than by their own mating success. Males may thus be constrained by their inability to perceive lek size accurately prior to female arrival leading to suboptimally large leks in which male mating success is ultimately reduced (e.g. Neems *et al.* 1992). This mechanism would be reinforced if energetic costs involved in locating or establishing position in another lek are high (Regelmann 1984, Vehrencamp *et al.* 1989, Neems *et al.* 1990, Alatalo *et al.* 1992, Höglund *et al.* 1993). In this situation, once in a large lek it may be less costly for a male to remain there and suffer reduced mating success than to spend time and energy relocating to a smaller lek.

Finally, the numbers of females per male caught in the traps may not be a true reflection of male mating success. Each sample collected represents the availability of females at an instantaneous point in time, rather than over the entire night. If the rate of copulations per female visit increases with lek size as in black grouse (Alatalo *et al.* 1992), the numbers of females present per male are not representative of a male's reproductive success. To test this hypothesis requires field data on copulation rates for individual males and female visits across leks of varying sizes.

Conclusion

In conclusion, the evidence presented in this chapter suggests that a hotspot process, coupled with female preference for larger leks, may be responsible for the evolution of the lek mating system of *L. longipalpis*. Without information regarding the mating success of individual males, however, it is not possible to distinguish further between the proposed models.

Chapter 5

Age related differences in male mating success and fertility

Summary

1. Age related differences in male reproductive success have been reported for several species. Traditionally, female preference for older males has been explained in terms of viability indicator models of sexual selection. However, females could also gain direct benefits such as increased probability of fertilisation. In this chapter I examine how female choice and male fertility vary with male age in the phlebotomine sandfly, *Lutzomyia longipalpis*.
2. In the first experiment I explored the relationship between male age and fertility. Females were randomly allocated to a male selected from one of three age categories (young: 12hr-2d; middle-aged: 4-6d; old: 8-10d) and then differences in fertility were compared.
3. Females randomly allocated to young and middle-aged males gained a direct fitness benefit from their mate: proportionally more eggs laid hatched as first instar larvae.
4. To investigate whether male mating success was age-related, females were permitted a choice of six males consisting of two males selected from each of the age categories. The average reproductive success and differences in fertility of each age category were then compared.
5. Females permitted a free choice of male preferentially mated with middle-aged males, but there were no differences in average fertility across the three age categories.
6. The experiment is one of the first to show that female discrimination between males may be related to differences in fertility. It also provides contradictory evidence against viability indicator models of sexual selection. Possible mechanistic and evolutionary explanations for the observed age-related variance in male reproductive success and fertility are discussed.

Introduction

Female preferences for older mates have been widely documented, in species with both resource-based breeding systems (Burley 1981, Woodhead 1986, Zuk 1987, Conner 1989, Komers and Dhindsa 1989, Simmons and Zuk 1992, Enström 1993, Simmons 1995, Wagner *et al.* 1996, but see Burley and Moran 1979) and in non-resource based systems, where a female ostensibly obtains genetic material from her choice of mate (Weatherhead 1986, Alatalo *et al.* 1986, Clutton-Brock *et al.* 1988, Kruijt and de Vos 1988, Clutton-Brock and Albon 1989, McDonald 1989, Sundberg and Dixon 1996, Widemo 1996). A potential problem in studies of age-related female choice is that age may be correlated with other characters (Woodhead 1986, Clutton-Brock 1988, Simmons 1988, Borgia and Collis 1989, Côté and Hunte 1993, Sundberg 1994, Sundberg and Larsson 1994, Simmons 1995, but see Petrie 1993). Moreover, the interpretation of the pattern of preference may be confounded if, for example, a male's competitive ability is age dependent and a determinant of his mating success. For example in birds, the reproductive success and the ability of male black grouse, *Tetrao tetrix*, to retain a territory increase over the first four years of life, but then decline as males become less able to compete for territories (Kruijt and de Vos 1988). Similarly, in red deer, *Cervus elaphus*, the reproductive success of males increases up to approximately 10 years of age, but then declines rapidly due to reduced competitive ability (Clutton-Brock and Albon 1989).

Traditionally, female preferences for older mates have been explained in terms of viability indicator models of sexual selection: older males have shown their ability to survive and so are more likely to pass on good genes for survival or parasite resistance to their offspring (Trivers 1972, Halliday 1978, Manning 1985, Hamilton and Zuk 1982, Borgia and Collis 1989). These explanations were founded principally on verbal arguments, but two sets of workers have recently attempted to provide a theoretical framework for these empirical observations (Hansen and Price 1995, Kokko and Lindström 1996a). From quantitative genetic models of life history evolution, Hansen and Price (1995) argue that old males are

in fact rarely genetically superior to younger males. They suggest that because of the history of selection on their parents, younger males are born from a better adapted gene pool. Also, germline mutations, which are generally deleterious, significantly decrease the breeding fitness of an individual throughout its life-span. Kokko and Lindström (1996a) challenged this view in a series of models of female preference for older males in constantly changing environments. They provide theoretical support for viability indicators or "good genes" models of sexual selection and propose that the mutation-selection balance is sufficient to maintain female choice for older mates via genetic effects alone.

While indirect selection alone may maintain female choice for older males, it is possible that such preferences also confer an immediate fitness benefit on choosy females, such as assurance of fertilisation (Reynolds and Gross 1990, Williams 1992, Sheldon 1994). Age-related differences in fertility have been reported in a few species. In *Drosophila melanogaster*, males permitted to mate with females continually throughout their lifetime suffer a decline in fertility with age (Partridge 1988). Conversely, in a fungus beetle, *Bolitotherus cornutus*, older males have higher insemination success in the laboratory (Conner 1989). In crickets, choice is paradoxical as females prefer to mate with older males (Zuk 1987, Simmons and Zuk 1992), despite the fact that older males are less fecund (Simmons 1988). In this study, I examine how female choice and male fertility vary with male age in *Lutzomyia longipalpis*.

Laboratory experiments on *L. longipalpis* leks made up of similarly aged males (4-6 d) found that male reproductive success is skewed, with a high degree of unanimity in female choice resulting in a few males obtaining a large proportion of available matings (Chapter 3). In the field, males return to leks on successive nights (Kelly *et al.* 1996), so that females presumably have an opportunity to select between males differing in age. The only other previous laboratory study (Jarvis and Rutledge 1992) of *L. longipalpis* found that in the presence of a host, male age did correlate with mating success: middle-aged males (6-10d) achieved the greatest proportion of available matings when in competition with young (1-5d) and old

(11-15d) males. Moreover, Jarvis and Rutledge presented evidence to suggest that females mated to older males laid more eggs than those mated to younger males, however there was no difference in the number hatched and they were only able to compare young and old males. A further problem in their study was that they measured male mating success by the transfer presence of fluorescent dye on the female's abdomen. This may be misleading as dye may be transferred from a male even if a female rejects a male prior to the termination of copulation (Chapter 3).

To examine the relationships between age, female choice and male fertility, I made two changes to the structure of the age classes used by Jarvis and Rutledge (1992). First, I created a distinct age gap between categories, and within each age category males differed by a maximum of 2d (cf. 5d). Second, to take account of my own laboratory data on survival (Chapter 2), the age categories were lowered as follows - young males: 12hr-2d; middle-aged males: 4-6d; old males: 8-10d. In addition, as the numbers of eggs laid are dependent on the size of bloodmeal taken by a female (Ready 1979) and females are reluctant to feed individually (pers. obs.), I bloodfed females prior to experimentation and then selected only those with fully extended abdomens for use in experiments. Here I present the results of two experiments. In the first, I examined male fertility in relation to age in the absence of female choice. This was achieved by allocating single males of varying ages to virgin females and comparing the fertility of males from each category. In the second I assessed whether, when presented with males differing in age, female mate choice was age-dependent. I explored differences in male fertility as in the first experiment.

Methods

Unless otherwise specified, all flies used in the experiments were separated and placed into single-sex cages at less than 12hr after eclosion and maintained in low numbers (<30) under standard laboratory conditions (Chapter 2). To eliminate potential cage effects, most cages contributed on different days to more than one

age group. All females used in the trials were bloodfed on an anaesthetised hamster, 24hr prior to experimental use (see Chapter 2).

(i) Male age and fertility

To explore how a male's fertility varied with age, 225 females were allocated a single male from a randomly chosen age group. 75 virgin males were selected from each age group and individually exposed to 4-6d old virgin females drawn from a single-sex stock cage. Females were fed 24hr earlier (see Chapter 2). Each pair was introduced into a net cage and left until they copulated.

To investigate the consequences of a female's allocated mate on her fecundity, mated females were individually placed in oviposition tubes for egg laying and monitored daily until death. All eggs laid were then counted and transferred to a Petri-dish for egg hatch. The abdomen of the female was dissected to check for any retained eggs. Petri-dishes were checked daily for signs of egg hatch and the total number of broken egg shells were counted as a measure of fecundity.

(ii) Age and female choice

To assess whether mating success was related to age in *L. longipalpis*, females were permitted a choice of six males, consisting of two drawn from each of three age classes: young (12hr-2d), middle (4-6d) and old (8-10d). In total 16 such sextets were set up, each made up of males from any appropriately aged stock cage. The males of each age class were marked with one of three fluorescent dyes (pink, green or blue), known to have no effect on mating success (Chapter 3). The dye colour used for each age class was rotated between trials. Marked sets of males were placed in net cages and permitted a 10min acclimatisation period, after which 10 virgin females were sequentially added to each set of six males. For each female, the age class of the male she eventually mated with, and the length of copulation were noted and the females then removed prior to the introduction of the next female. To investigate the consequences of a female's choice of mate on her fecundity, female fecundity was monitored as above.

All measures of fecundity (numbers of eggs laid and retained, numbers of eggs hatched and proportion of eggs hatched) and the length of copulation were compared using nonparametric statistics as transformation of the data did not sufficiently improve its normality. The 95% significance level after correction for five multiple comparisons (Chapter 2) is given by $P < 0.01$. The proportion of matings obtained by males of different age classes were compared using a logistic regression, specifying a Binomial error distribution. Male age class and trial were added as factors with 3 and 16 levels respectively. The minimal adequate model was scaled to correct for overdispersion and the significance of each parameter was determined as described in Chapter 2.

Results

(i) Male age and fertility

All females mated with the assigned male irrespective of his age. Time spent in *copula* and the number of eggs laid, retained and hatched were all independent of male age (Table 5.1). There was, however, a significant difference across sire age classes in the proportion of eggs hatched (Table 5.1, Figure 5.1). A significantly lower proportion of eggs hatched from females mated to old age males than from those females mating with young or middle-aged males (Figure 5.1). The proportion of eggs hatching was, however, similar for females mating with middle-aged or young males.

Table 5.1. Median (interquartile range) of copulation and numbers of eggs laid, retained and hatched by females mated to young, middle-aged or old males. Uncorrected significance levels are given by: NS = not significant; ** = $P < 0.01$.

	Age class of mating male $n = 75$ females			Kruskal-Wallis test
	Young	Middle-aged	Old	H_{corr}
Copulation length in seconds	35.0 (30.3-45)	38.0 (31-49)	37.0 (31-48)	2.13 NS
Number of eggs laid	54.0 (12.3-60.8)	49.0 (35-60)	52.0 (0-68)	0.76 NS
Number of eggs retained	0.0 (0)	0.0 (0)	0.0 (0)	0.64 NS
Number of eggs hatched	41.0 (0-55.8)	43.0 (28.5-54)	40.0 (0-56.3)	2.06 NS
Proportion of laid eggs hatched	0.87 (0-0.95)	0.91 (0.79-0.98)	0.76 (0-0.83)	24.1 **

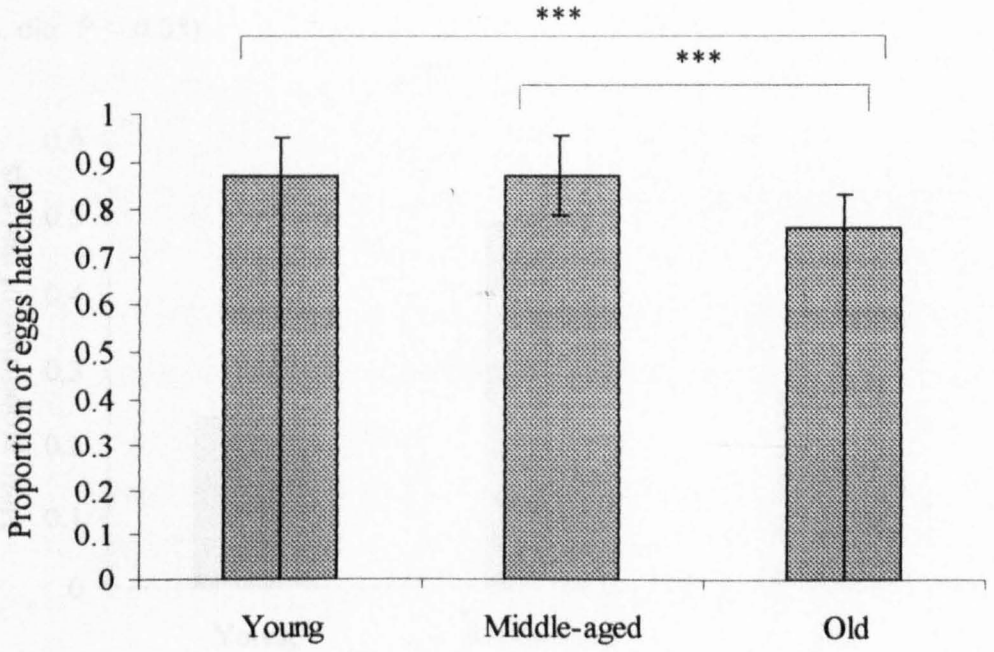


Figure 5.1. Median proportion of laid eggs hatching as first instar larvae in relation to sire age. Error bars represent the upper and lower quartiles. Significance levels for comparisons between categories given by *** = $P < 0.001$.

(ii) Age and female choice

When presented with young, middle-aged and old males, females consistently preferred to mate with middle-aged males ($F_{2,47} = 7.30$, $P < 0.05$, Figure 5.2), irrespective of trial ($F_{15,47} = 0.00$, NS). Males of all ages approached and wing-fluttered at females, although this was not quantified. Among those males that were successful, the length of time spent in *copula* did not vary with age (Table 5.2).

Female fecundity was also independent of male age (Table 5.2). Females mating with middle-aged males did not lay or hatch more eggs than those females mating with young or old males. The proportion of eggs laid which hatched into first instar larvae was comparable across age classes. There were, however, significant differences between the numbers of eggs retained across females: females mating with old males retained more eggs than those females mating with middle-aged or young males (Kruskal-Wallis multiple comparisons between treatment groups: young vs. middle-aged: NS; young vs. old: $P < 0.05$; middle-aged vs. old: $P < 0.05$).

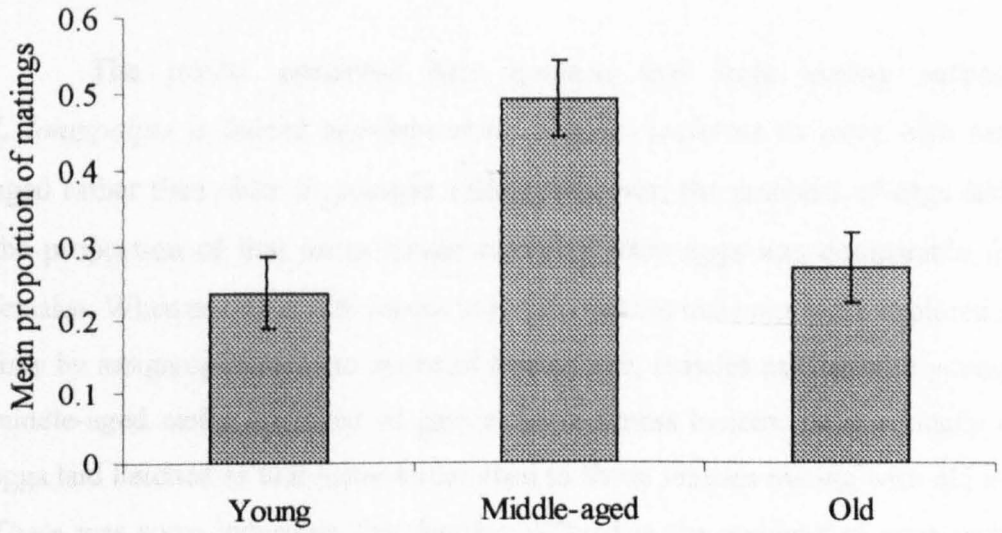


Figure 5.2. Mean proportions (± s.e.) of matings obtained by males in young, middle-aged and old age classes when females are permitted a free choice of mate. Data are from 16 trials each involving 10 females.

Table 5.2. Median (interquartile range) lengths of copulation, and numbers of eggs laid, retained and hatched by females permitted a free choice between young, middle-aged and old males. Notation as in Table 5.1.

	Age of mating male			
	Young $n = 39$ females	Middle-aged $n = 80$ females	Old $n = 41$ females	Kruskal-Wallis test (H_{corr})
Copulation length in seconds	48.0 (36.3-65)	47.0 (38-65.5)	46.0 (40-55.3)	0.33 NS
Number of eggs laid	49.0 (27.8-56.8)	45.5 (17-59)	57.0 (40.3-64.3)	4.58 NS
Number of eggs retained	0.0 (0-1.75)	0.0 (0)	0.0 (0)	9.39 **
Number of eggs hatched	37.0 (17-49)	33.0 (3.5-50)	41.0 (12-50.3)	1.33 NS
Proportion of laid eggs hatched	0.79 (0.43-0.9)	0.66 (0.13-0.88)	0.73 (0.25-0.85)	1.82 NS

Discussion

The results presented here confirm that male mating success in *L. longipalpis* is indeed age-dependent: females preferred to mate with middle-aged rather than older or younger males. However, the numbers of eggs laid and the proportion of first instar larvae emerging from eggs was comparable for all females. When potential differences in fertility across male age were explored more fully by assigning females to males of known age, females mating with young and middle-aged males appeared to gain a direct fitness benefit: proportionally more eggs laid hatched as first instar larvae than to those females mating with old males. There was some indication that females differed in the numbers of eggs retained, but this may simply reflect the high levels of mortality observed during oviposition in the laboratory (Killick-Kendrick *et al.* 1977).

What are the possible mechanisms for the observed age preference? In subsequent work the potential importance of wing-fluttering and pheromones in female mate choice among middle-aged males are highlighted (Chapter 7): females prefer to mate with males that produce more pheromone and wing-flutter more. It is probable that this is also applicable across age groups. While there is no available data on age-related investment in wing-fluttering in male *L. longipalpis*, if a male's ability to wing-flutter declines with age, as has been found with behavioural characteristics such as courtship and male competitive success in other species (Clutton-Brock and Albon 1989, Kruijt and de Vos 1988, Widemo 1996), older males may be less able to attract females. However, this does not explain why females did not mate equally often with young and middle-aged males. It is possible that females discriminated against young males because they were not as efficient at wing-fluttering, however they may also have been constrained physiologically. While young males (<4d) are sexually mature (Chaniotis 1967, pers. obs.) they are not capable of pheromone production (Boufana *et al.* 1986) and thus they may have been restricted in the cues they could have used to attract females. It is not known whether pheromone production declines in older flies, however this would further explain why this category was less successful.

Mechanisms aside, in evolutionary terms why did females preferentially mate with young or average aged males when theoretical predictions suggest that females should prefer older males of proven survival ability (Trivers 1972, Halliday 1978, Manning 1985, Kokko and Lindström 1996a, but see Hansen and Price 1996)? My data indicate that age-related preferences may enable females to gain direct benefits through increasing their probability of fertilisation (see also Hansen and Price 1996). In the following chapter, exploring the fitness consequences of female choice between males of the same age (middle-aged), I found evidence that choice within age groups conferred a very different benefit on choosy females: females mating with attractive males gained indirect benefits by producing more attractive sons (Chapter 6). In the present experiment, looking at choice across age groups, I did not examine whether sons of differently aged sires differed in their attractiveness, nor whether offspring were better able to survive, thus these can not

be ruled out as further potential benefits (in addition to increased likelihood of fertilisation) of age-based mate choice.

A further question raised by this study is how did the observed differences in fecundity arise? There are at least two possible explanations: males of different ages may vary in their ability to transfer sperm successfully (Woodhead 1986), or they may vary in the quality of their sperm or spermatophores (Markow 1988, Markow *et al.* 1990). In *L. longipalpis* the length of time spent in copulation was similar for all matings observed (*cf.* cockroaches - Woodhead 1986). This suggests that males did not differ in their ability to transfer sperm, and that differences in sperm quality may be more important. One reason for a decrease in sperm quality with increased age could be that older males suffer the effects of deleterious germ-line mutations as suggested by Hansen and Price (1996).

A potential problem in the experiment is that I was unable to control for differential maternal investment (Burley 1988). It is possible that females that mated with a young or middle-aged male invested more heavily in offspring production thereby increasing the proportion of eggs hatching. While I cannot refute this suggestion completely, two lines of evidence suggest that differential maternal investment is unlikely to have occurred in this study. First, in species which lay large quantities of eggs it might be expected that differential investment would manifest itself in terms of quantitative rather than qualitative differences (Simmons 1987). In the cricket, *Gryllus bimaculatus*, females randomly allocated large, preferred males laid significantly more eggs than those females allocated smaller unpreferred males (Simmons 1987). In contrast, in this study, I found no differences in the numbers of eggs laid by females. Second, females allocated mates of different ages may be expected to allocate more resources to the offspring of males which they choose themselves than to those males with preferred phenotypes to which they are allocated randomly (Simmons 1987). Contrary to this prediction, in this study the proportions of eggs hatched from females permitted a choice of mate were consistently lower than the proportions of eggs hatched from those females randomly allocated a male (compare Tables 5.1 and Table 5.2).

Conclusion

In conclusion, this experiment shows that in *L. longipalpis* male mating success is age-dependent and age is at least partially correlated with a male's ability to fertilise a female. It is still unclear how these results relate to the field, where females are likely to encounter males differing in both age and mating experience. At present, age-related studies on field caught male flies are not possible because in contrast to females, there is no successful method of ageing them (Dye *et al.* 1991). One final important caveat here is that, as in several other studies (Woodhead 1986, Zuk 1987), all males used were inexperienced virgins. An advantage of using inexperienced males is that the potential effects of cumulative deleterious germ-line mutations are not confounded by male investment in early-age reproduction (Hansen and Price 1995, Kokko and Lindström 1996a). In the field, however, older males are likely to have experienced a number of matings. This may have consequences both in terms of their fertility and possibly for any potential direct benefits accruing to choosy females. In addition, female preference could conceivably be influenced by previous male experience (Markow *et al.* 1978, Nakatsuru and Kramer 1982). Thus the wider significance of the present results can only be fully elucidated with additional work on experienced flies.

Chapter 6

An experimental investigation of the fitness consequences of female choice

Summary

1. The fitness consequences of female mate choice were investigated to test the relative importance of three competing, but not mutually exclusive, hypotheses proposed to explain the maintenance of strong mating preferences in lek-breeding species.
2. I allowed females to mate freely in aggregations of five males that differed in their attractiveness to females and then compared the survival and fecundity of females with the attractiveness of their mate. I then reared the offspring of these individual females through to adults and compared the survival of offspring of both sexes, the fecundity of daughters and the mating success of sons with either the absolute or the ranked mating success of their father. I used these results to test simultaneously the predictions of the three models.
3. Weak evidence is presented to support direct benefits models: females mating with generally preferred males survived longer after oviposition than females mated to less preferred males. Total female longevity, however, did not increase with mate attractiveness and the number of eggs laid was comparable across all females.
4. In contrast to the predictions of good-genes models, I found no evidence to suggest that offspring sired by more successful males survived longer than those sired by less successful males. Neither were daughters sired by generally preferred males more fecund.
5. The results supported the prediction of Fisherian models: sons sired by more attractive fathers were themselves more attractive to females than those sired by less attractive males.
6. It is proposed that, in the absence of unequivocal support for the other hypotheses tested, the experiment provides evidence to support a Fisherian type mechanism being at least partially responsible for maintaining female mating preferences in this lek-breeding insect.

Introduction

Despite increasing empirical evidence of it's widespread significance, the evolution of female choice remains poorly understood (Kirkpatrick 1982, Kirkpatrick and Ryan 1991, Andersson 1994). In species where females gain immediate fitness benefits from mate choice, such as a good quality territory or high quality parental care, the selective advantages of mate choice are clear. Mate choice benefits are less apparent on leks, where by definition males provide no material resources or parental care to choosy females (but see Robertson 1990, Kirkpatrick and Ryan 1991). Moreover, in lekking species, variance in male reproductive success is often extreme, with sexual dimorphism being more pronounced and females more discriminating than in other mating systems (Bradbury *et al.* 1985, Reynolds and Gross 1990, Wiley 1991, Oakes 1992, Höglund and Alatalo 1995, Widemo and Owens 1995). The existence of apparently strong mating preferences in the absence of direct benefits has been termed the "paradox of the lek" (Taylor and Williams 1982, Rowle and Houle 1996). A series of competing, but non-exclusive hypotheses have been proposed to explain how female preferences might be maintained even in lek-breeders. These can be divided into direct and indirect benefits models, depending on the form of the payoff accruing to discriminating females (for reviews see Kirkpatrick and Ryan 1991, Andersson 1994, Andersson and Iwasa 1996).

Direct benefits models

These models suggest that a female gains immediate benefits from her choice of mate in terms of increased survival or fertility (Kirkpatrick and Ryan 1991, Price *et al.* 1993). Empirical evidence from resource-defence mating systems has shown that large nutrient rich nuptial gifts, access to defended resources, high quality oviposition sites, increased levels of paternal care, more viable sperm, reduced levels of sexually transmitted diseases, or the ability to protect a female from harassment by other males, are all qualities favoured in female choice (Campanella and Wolf 1974, Wrangham 1980, Burley 1981, Partridge and Farqhuar 1983, Ryan 1983, Simmons 1988, Clayton

1990, Gilburn *et al.* 1992). It is even proposed that the lek paradox may in fact be more apparent than real, because a female mating on a lek could conceivably accrue immediate benefits from her choice of male through reduced risks of predation, male harassment, infertile matings or infection with a sexually transmitted disease (Reynolds and Gross 1990, Kirkpatrick and Ryan 1991). However, at present there is little empirical evidence to support this hypothesis (for discussion see Höglund and Alatalo 1995).

Indirect selection models

These models suggest that discriminating females benefit indirectly from their choice of mate by increasing the potential reproductive success of their offspring. The models require that the female preference and the preferred male trait are heritable, and that they covary across generations (Lande 1981, for a review see Andersson 1994). Current literature focuses on two non-exclusive mechanisms of indirect selection: the Fisherian process and good-genes models.

Fisherian process - The Fisherian process was verbally proposed by Fisher (1915, 1930, 1958) and later formalised by a variety of workers (O'Donald 1962, Lande 1981, Kirkpatrick 1982, Pomiankowski *et al.* 1991). Fisherian models suggest that a female preference for a particular male trait may arise in the population as a result of random mutation (Fisher 1915, 1930, 1958). (Although, note that sensory bias models suggest that a preference for a particular trait may arise because of male exploitation of a female's pre-existing sensory bias [Arak and Enquist 1993, Basolo 1995, Basolo and Endler 1995, Johnstone 1995]). Regardless of its origin, this preference in turn confers a mating advantage on males bearing the preferred trait. Provided that there is genetic variation in the female preference and the male trait, females carrying a gene for preferring a particular male phenotype will produce sons carrying genes for both the preferred trait (which they express) and (albeit unexpressed) the genes for its preference. Because these sons will in turn enjoy disproportionate reproductive success through being chosen by other choosy females,

the frequency of genes for both the trait and the preferences will increase. This ability of the preference genes to select, indirectly, copies of themselves thereby drives a positive feedback process, with females becoming progressively choosier and male traits progressively more exaggerated over time, until the cost of the trait exactly balances its mating advantage (Lande 1981, Arnold 1983, Andersson 1994). Fisherian models therefore predict heritability of male attractiveness, with the sons of preferred males themselves having high mating success. In contrast to good-genes models and contrary to Fisher's initial hypotheses, the mechanism does not require that preferred male traits are indicators of heritable variation in viability (Lande 1980, 1981, Kirkpatrick 1982, Pomiankowski 1988).

Empirical evidence supporting the Fisher process is limited (see Bakker and Pomiankowski 1995). Several studies report heritability of the preferred male trait (Hedrick 1988, Bakker 1993, Houde and Endler 1990, Bakker and Mundwiler 1992, Gilburn *et al.* 1993, Houde 1994, Wilkinson and Reillo 1994), however this is a common prediction of all models of indirect selection. Only one study in the Mediterranean fruit fly, *Ceratitus capitata,* has investigated and failed to find supporting evidence for the explicit prediction that sons of attractive males are preferred over those of less attractive males (Whittier and Kaneshiro 1995). In addition, while Whittier and Kaneshiro (1995) also tested for potential direct benefits accruing to females mating with highly successful males, none of the above studies has demonstrated heritability of the preferred trait in the absence of all other potential fitness payoffs.

Good-genes models - Good-genes or viability indicator models of female choice suggest that mating preferences evolve because they facilitate the identification of mates of high genetic quality (Zahavi 1975, Hamilton and Zuk 1982, see also Pomiankowski 1988 for a review). The models state that if male traits are differentially expressed in relation to the condition of the male, a well-developed trait should indicate high viability. Provided that the male trait, male viability and female preference

all have a heritable component, a female choosing a highly ornamented male will benefit indirectly by producing more viable offspring of both sexes. The central prediction of good-genes models is thus that males bearing the most elaborate traits will produce more viable sons and daughters.

Most recent empirical tests of the good-genes mechanism have sought a correlation between the preferred male trait and male viability. For example, Milinski and Bakker (1990) report a negative correlation between the intensity of red coloration and parasite load in the stickleback, *Gasterosteus aculeatus*, and similar correlations have been reported in other species (Møller 1990, Zuk *et al.* 1990, von-Schantz *et al.* 1992, Zuk *et al.* 1992). While these studies infer the presence of a good-genes mechanism, they fail to explore heritability of the preferred trait and thus viability. Early support for enhanced offspring viability came from Partridge (1980) who showed *Drosophila melanogaster* females mated in cages containing several males produced fitter larvae than females mated with males randomly selected from the population (but see Schaeffer *et al.* 1984). However, females were exposed to different densities of males and more importantly male-male interactions could not be excluded as a factor determining male mating success. By using cross-fostering techniques and randomly allocating females to males of known attractiveness Petrie (1994) found that in the peafowl, *Pavo cristatus*, females mating with preferred males produced offspring which survived longer than offspring sired by unpreferred males. Similar results are reported in great tits, *Parus major*, (Norris 1993) and in pheasant, *Phasianus colchicus*, (von Schantz *et al.* 1994). As with tests looking for a Fisherian mechanism, a limitation of all these studies reporting good-genes effects is that they do not exclude alternative potential benefits of female choice which may be operating simultaneously.

To my knowledge, no study has tried to estimate the relative importance of all three types of models proposed to explain the evolution of female mate choice simultaneously. Here, I attempt to do so by examining the consequences of a female's

mate choice in terms of her own fitness and that of her offspring. I allowed females to mate freely in aggregations of five males from the preferred age class (Chapter 5) that differed in their attractiveness to females and then compared the survival and fecundity of females with male attractiveness. I then reared the offspring of these individual females through to adults and compared the survival of offspring of both sexes, the fecundity of daughters and the mating success of sons with the mating success of their father. I used these results to test the following predictions of the three models:

(i) if females gain direct mate choice benefits, those mating with generally preferred males should survive longer and/or be more fecund than females mating with less attractive males;

(ii) if good-genes models hold, then both the sons and daughters of generally preferred males should exhibit higher survival than those offspring sired by less preferred fathers; daughters of the most attractive males should perhaps have increased fecundity as well;

(iii) finally, under Fisherian models, sons sired by generally preferred males should be more attractive to females than sons of less popular fathers.

Methods

Stock flies

All flies used in this study were maintained in single-sex stock cages under standard rearing conditions (Chapter 2) for 4-6d prior to use.

(i) Male mating success

The aims of the first stage of the experiment were to assign females to males differing in overall mating success and to explore potential correlates of female choice. A total of 25 sets of five males were randomly selected from the stock cage, individually marked with fluorescent powder (Chapter 2) and released to form leks in net cages. Marking has no impact on a male's immediate survival or mating success in

the laboratory (Chapters 2 and 3). After a 10min acclimatisation period, 10 females, fed 24hr earlier, were selected from a stock cage and sequentially exposed to one set of males. Each female was observed continually after introduction into the cage and removed after copulation occurred. The identity of the successful male was noted and 10min later a further female introduced. This first set of trials thus produced 250 females that had mated with males of high or average attractiveness (see below). In order to generate a group of females that had mated with consistently unattractive males, those males that obtained the fewest matings (usually zero, sometimes one) from these first trials were then regrouped and used for a further set of six mating trials, each with 10 females and five previously unattractive males. Conditions were identical in both sets of trials and all females used in subsequent analyses had chosen their mates freely, whether they mated with males of high, average or low attractiveness. Note also that the mating success of fathers was defined as either the absolute or the relative number of matings achieved by males in their first trial, and so for unattractive males this was unaffected by their success in their second trial. Males may mate with at least six females without suffering from reduced fertility (Chapter 2). In order to eliminate any potentially confounding effects of sperm depletion, I therefore adopted a conservative rule of discarding any females that were not among the first four to mate with a given male.

After each trial, males were stored in alcohol and were later measured to explore potential morphological correlates of mating success (see Chapter 4 for details). Differences in sample sizes for various measures are due to damaged limbs.

(ii) Female survival and fecundity, and immature offspring survival

Mated females were individually placed in oviposition tubes and a swab of cotton wool soaked in 70% sugar solution placed on the lid. All tubes were maintained in a polystyrene-lined wooden cabinet kept at approximately 28°C, and 80% humidity. To avoid any effects of environmental variation within the cabinet, all tubes were moved daily to new locations. Females were monitored every day until death, to check

for signs of oviposition and survival. Three days after the start of oviposition, all eggs laid were counted and transferred to a small Petri-dish (5cm diameter, 1cm height), lined with moistened filter paper. Small Petri-dishes were used in order to maintain larval densities comparable to standard rearing conditions (*cf.* Chapter 2). After death the abdomen of the female was dissected under a binocular microscope and any remaining eggs were recorded. The Petri-dishes were monitored daily and after egg hatch, larval food (dried liver powder) was sprinkled sparingly over the dish and the filter paper was moistened as required. I recorded the numbers of larvae in each of the four instars, as well as pupae and eclosing adults. Numbers of first instar larvae were assessed by counting and removing broken egg shells. All other stages were counted directly. Any dish with fungal growth forming a cover over the dish, which may result in large losses of larvae (pers. obs.), was excluded from further statistical analyses.

Female survival was measured as (i) pre-oviposition survival - time in days from blood-feeding to the first day of oviposition, (ii) post-oviposition survival - time after the first day of oviposition to death, and (iii) total time to death. Female fecundity was assessed by scoring both the number of eggs laid and the number of eggs retained. Offspring survival was measured as the proportion of offspring surviving through successive instars and the proportion of eggs laid which resulted in eclosing adults.

Measures of maternal survival and fecundity and immature offspring survival were compared with paternal mating success. After correction for multiple comparisons (Chapter 2), the 95% significance level for measures of maternal survival and fecundity is $P < 0.01$ (five comparisons) and for immature offspring survival is $P < 0.006$ (eight comparisons).

(iii) Survival of adult offspring and fecundity of daughters

Because females are reluctant to feed individually (pers. obs.), and to facilitate comparison of sons sired by males varying in attractiveness, adult offspring were assigned to one of three groups depending on the relative attractiveness of their fathers

in the first set of trials. Individual paternity was no longer recorded. High-ranking fathers were defined as those obtaining the highest number of matings in a trial (mean number of matings [± s.e.] = 4.94 [± 0.16]); average-ranking fathers those males with median success (2.00 [± 0.09] matings); and low-ranking fathers as any male obtaining the lowest number of matings in the first trial (0.07 [± 0.01] matings). I rejected the adult offspring of any male not in these categories.

Because good-genes effects might manifest themselves either through higher offspring survival or increased fecundity of daughters, I made the following four comparisons across treatment groups: (i) survival of sons, (ii) survival of unfed daughters, (iii) survival of fed daughters, (iv) fecundity of fed daughters. A maximum of two offspring from each female that mated with high, average and low ranking males were randomly selected for each comparison.

For the first two comparisons, eclosing sons and daughters were individually placed in oviposition tubes and thereafter treated in the same way as their mothers (see above). For the third and fourth comparison daughters were placed in oviposition tubes on eclosion and at three days of age released in sets of up to five into a net cage together with an equal number of stock males and an anaesthetised host (Chapter 2). After 30min of observation (to ensure they all fed and copulated with a male), the fed daughters were reintroduced into the tubes and monitored daily until death for signs of oviposition. Any eggs laid were transferred onto Petri-dishes lined with moistened filter paper and the number of eggs hatching was recorded. Survival of both fed and unfed daughters and of sons was monitored daily and defined as the total number of days until death. In addition, for fed daughters, survival, pre- and post-oviposition were calculated and fecundity was measured as for their mothers. The proportion of eggs laid which hatched was also calculated.

For all adult offspring, the total numbers of days survived was compared across paternal mating rank. A Weibull distribution model was employed to generate

predicted survival curves, then differences between paternal groups and sons and daughters were assessed using logistic models with a specified Poisson error distribution (see Chapter 2 for a description of the method; after Crawley 1993). Paternal rank (high, average, low) and offspring type (son, unfed daughter, fed daughter) were added as three level factors to the models. The significance of each parameter was determined by stepwise deletion as described in Chapter 2. For fed daughters, the numbers of days survived pre- and post-oviposition were low and did not permit meaningful comparison of survival curves, and so unfitted numbers of days were used for analyses of survival. Measures of fecundity were compared across paternal rank using Spearman rank correlations. After correction for six multiple comparisons the adjusted 95% significance level is $P < 0.008$.

(iv) Attractiveness of sons

The attractiveness of sons was assessed by measuring their mating success in triads consisting of a single son from each paternal rank (high, average, and low). Any son was only used in a single triad and all 38 triads had unique parentage. Each triad was introduced into a net cage 10min before the introduction of a virgin female. She was permitted to mate with the male of her choice whose identity was noted. The female was then removed and other females successively introduced until each triad had been exposed to a total of six females. Possible morphological correlates of the mating success of sons were measured in the same manner as for their fathers (see above).

Differences in son attractiveness between treatment groups were assessed using a logistic regression in GLIM 4.0. As data were proportional, a binomial error distribution was assumed with the number of matings obtained as the dependent variable and the number of females introduced per trial as the binomial denominator. Mean paternal mating success, calculated as the mean number of matings obtained by fathers in each rank, was added as a continuous variable into the model. Trial was added as a factor with 38 levels. The significance of the parameters was determined by

stepwise deletion as described in Chapter 2. Potential morphological correlates of a son's mating success were assessed by regressing morphological measurements on numbers of matings achieved. Son morphology was also compared with mean paternal mating success.

Results

(i) Male mating success

The frequency distribution of paternal mating success was significantly different from a computer generated random distribution in both the first and second mating trials (first trials: $\chi_5^2 = 106.2$, $P < 0.001$; second trials: $\chi_5^2 = 12.6$, $P < 0.05$), confirming previous observations (Chapter 3). However, no measure of male size was related to the numbers of matings achieved by a male (Table 6.1).

Table 6.1. Relationships between male mating success and male size.

Morphological character measured	Correlation between morphological character and mating success	*P* - value
Abdomen length	$r = 0.05, n = 96$	0.61
Left wing length	$r = 0.01, n = 81$	0.96
Right wing length	$r = 0.02, n = 79$	0.86

Numbers of females mating with males of high, average and low-ranking attractiveness are given in Table 6.2. This excludes 28 females which mated with attractive males who had already mated at least four times. The proportion of the remaining females surviving to oviposition was consistent across the groups

(χ_2^2 = 1.71, $P > 0.05$) and so further analyses were restricted to ovipositing females only.

(ii) Female survival and fecundity and immature offspring survival

Regression analyses showed that female post-oviposition survival increased with the mating success of their partners ($r = 0.22$, $n = 188$ females, $P = 0.002$; Figure 6.1). There was, however, no evidence to suggest that females mating with attractive males oviposited earlier or survived longer overall (numbers of days pre-oviposition: $r = 0.11$, $n = 188$ females, $P = 0.13$; total numbers of days prior to death: $r = 0.13$, $n = 188$ females, $P = 0.07$). Likewise, there was no correlation between either the number of eggs either laid or retained by a female and her mate's mating success (numbers of eggs laid: $r = 0.09$, $n = 188$ females, $P = 0.18$; numbers of eggs retained: $r_s = -0.13$, $n = 188$ females, $P = 0.07$).

Table 6.2. Numbers of ovipositing and non-ovipositing females contributing to individual analyses. Twenty-eight females choosing to mate with males that had already mated more than four times are excluded.

	Maternal status		Used for analyses of:
	Ovipositing	Non-ovipositing	
All females	188	89	Direct benefits to females Immature offspring survival
Females mating with a:			
High-rank male	58	40	Adult offspring survival
Average-rank male	36	13	Fecundity of daughters
Low-rank male	38	22	Attractiveness of sons

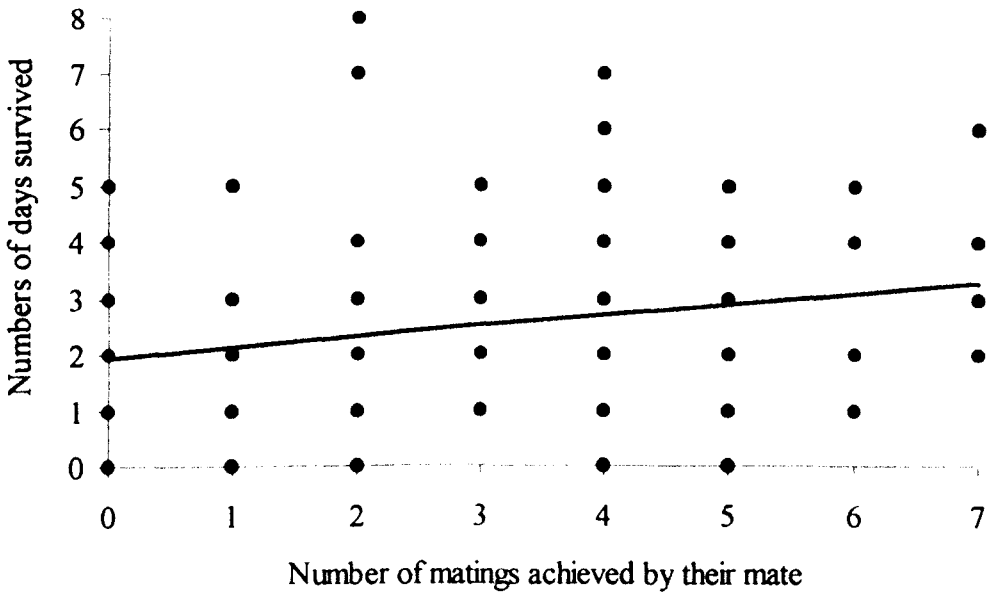


Figure 6.1. Relationship between numbers of days survived post-oviposition by females and the mating success of their partners.

Multiple comparisons revealed that offspring survival from egg hatch to adult eclosion was unrelated to paternal mating success (Table 6.3). In addition, there was no evidence that females differentially invested in sons or daughters as a function of paternal attractiveness: the female:male ratio of eclosing offspring was approximately unity (mean sex ratio [± s.e.] = 1.1 ± 0.07, χ^2=0.004, n = 153 females $P > 0.05$) and did not vary with paternal mating success (r_s = -0.05, n = 153 females, $P > 0.05$). Immature offspring survival in this experiment was comparable with the survival of larvae under standard laboratory conditions observed in Chapter 2 (G-test comparing survival of successive instars here and in Chapter 2 trial: χ_5^2 = 1.46, $P > 0.05$).

Table 6.3. Relationships between the proportions of offspring surviving through successive instars and paternal mating success.

Stage of the life-cycle	Correlation between proportion surviving and paternal mating success (*n* females)	*P* - value
Eggs - 1st instar	r_S = 0.12, (185)	0.18
1st - 2nd instar	r_S = -0.01, (173)	0.18
2nd - 3rd instar	r_S = -0.06, (167)	0.41
3rd - 4th instar	r_S = -0.01, (166)	0.95
4th - pupa	r_S = 0.06, (162)	0.48
Pupae - adult	r_S = 0.004, (159)	0.19
Egg - adult	r_S = 0.01, (159)	0.82
Total adults	r_S = 0.05, (159)	0.50

(iii) Survival of adult offspring and fecundity of daughters

Initially, survival data from all sons, unfed daughters and fed daughters were compared using the Weibull distribution model to predict differences between the three treatments (sons, unfed and fed daughters) and across paternal mating rank. The model predicts that the probability of death increases with age ($\alpha = 2.18$) and no evidence was found to suggest that offspring survival differed across paternal rank ($\chi_2^2 = 2.51$, $P > 0.05$). However, mean offspring survival varied significantly with sex and feeding status (mean days to death of sons = 11.5, unfed daughters = 8.2, fed daughters = 8.0, $\chi_2^2 = 90.25$, $P < 0.001$), suggesting that the predicted value of α may not be appropriate. Separate analyses were thus performed for sons, unfed daughters and fed daughters. Despite a weak trend suggesting that those daughters sired by low ranking fathers tend to survive longer, when corrected for multiple comparisons, the

mean number of days survived for all offspring was independent of paternal rank (Table 6.4, Figure 6.2a-c).

Table 6.4. Comparison of mean numbers of days survival of offspring from different paternal ranks, using a Weibull distribution model fitted in GLIM 4.0 (after Crawley 1993).

Offspring type	Mean (±s.e.) days survival across paternal rank			χ_2^2	Uncorrected *P*- value
	High (*n*)	Average (*n*)	Low (*n*)		
Sons	11.3±0.08 (116)	10.8±0.15 (70)	10.8±0.14 (76)	1.32	> 0.05
Unfed daughters	8.8±0.13 (116)	10.1±0.13 (72)	9.1±0.12 (70)	5.49	> 0.05
Fed daughters	7.94±0.15 (46)	8.37±0.25 (24)	9.03±0.23 (31)	6.50	< 0.025

Multiple correlations revealed that fed daughters sired by high ranking males did not oviposit sooner or survive longer post-oviposition than daughters sired by average or low ranking males (Table 6.5). Similarly, the number of eggs laid and retained and the proportions of eggs hatched were all unrelated to paternal rank (Table 6.5).

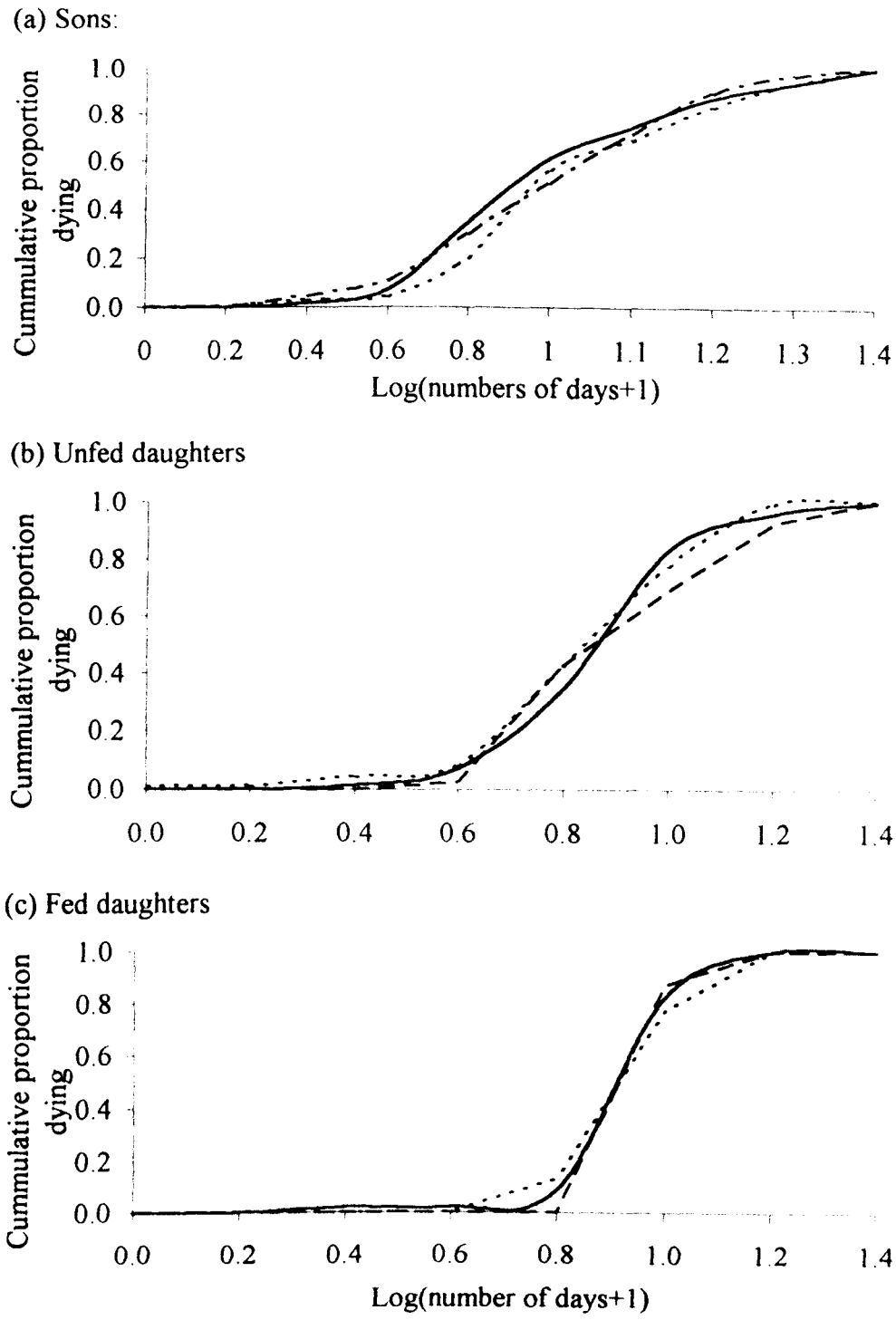


Figure 6.2. Proportion of offspring sired by high (——), average (– – –) and low (· · · ·) ranking males dying each day plotted against log(number of days+1). (a) sons; (b) unfed daughters; (c) fed daughters.

Table 6.5. Relationship between the survival and fecundity of daughters and paternal mating rank.

Parameter measured	Correlation between survival and fecundity of fed daughters and paternal rank (n = 101 females)	P - value
Days to oviposition	$F = 1.64$	0.20
Days post oviposition	$F = 1.02$	0.37
Number of eggs laid	$F = 1.87$	0.16
Number of eggs retained (✞)	$H = 4.05$	0.13
Prop. eggs hatched (✞)	$H = 1.96$	0.37

(✞) Compared using Kruskal-Wallis one-way analysis of variance as the distribution of data was non-normal.

(iv) Attractiveness of sons

The distribution of matings across the 38 triads involving sons was again significantly non-random (comparison with random distribution, see Chapter 3 for details: $\chi_3^2 = 12.72$, $P < 0.01$) with disproportionately high numbers of sons achieving greater than average or zero matings. After correction for overdispersion (after Crawley 1993), a logistic regression revealed a significant positive relationship between the mating success of a son and the average mating success of the group to which his father belonged (mean proportion of matings by sons [± se]: high = 0.42 [± 0.05], average = 0.32 [± 0.04], low = 0.28 [± 0.04]; $F_{1,113} = 6.24$, $P < 0.04$, Figure 6.3). There was no evidence to suggest that the distribution of matings between groups differed across trials ($F_{36,112} = 0.0$, $P > 0.05$). Finally, the size and wing length of sons were consistently unrelated to the numbers of matings they achieved (Table 6.6) or to the mating success of their fathers (Table 6.7).

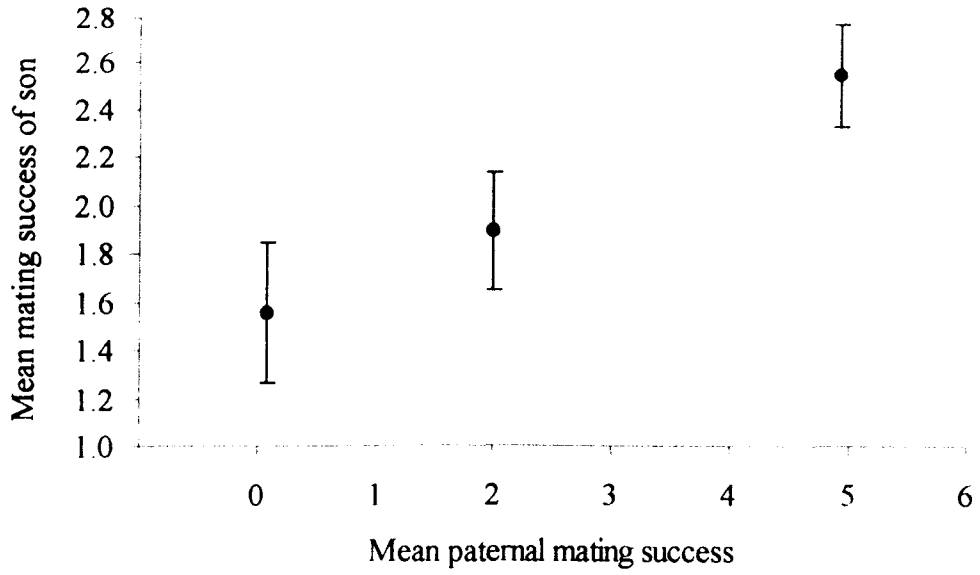


Figure 6.3. Relationship between the mean mating success of sons and the mean mating success of the group to which their fathers belonged. Error bars represent ± 1 standard error.

Table 6.6. Relationships between the mating success of sons and their morphology.

Morphological character measured	Correlation between morphological character and mating success	*P* - value
Abdomen length	$r = 0.01$, $n = 78$ males	0.21
Left wing length	$r = 0.01$ $n = 74$ males	0.42
Right wing length	$r = 0.004$, $n = 72$ males	0.61

Table 6.7. Relationships between the morphology of sons and the mean mating success of their fathers.

Morphological character measured	Correlation between morphological character and mating success	*P- value*
Abdomen length	$r = 0.07$, $n = 78$ males	0.52
Left wing length	$r = 0.06$, $n = 74$ males	0.25
Right wing length	$r = 0.11$, $n = 72$ males	0.35

Discussion

For female choice to be adaptive, discriminating females must accrue some benefit (Kirkpatrick and Ryan 1991, Andersson 1994). In lek-breeding *L. longipalpis*, female choice led to pronounced skew in male mating success and females mating with males that achieved high mating success survived longer post-oviposition than other females. Despite this, the mean overall survival and fecundity of females were independent of the mating success of their mate - thus there was no evidence of a net direct benefit of mate choice. Moreover, choosy females did not benefit indirectly by producing more viable offspring - measures of offspring survival and fecundity were consistently unrelated to paternal success. However, evidence is presented to suggest that females may benefit indirectly from mate choice via heritability of male attractiveness: sons sired by high ranking fathers themselves achieved more matings than sons sired by average or low ranking fathers. What do these observations reveal about the evolution of female choice in this species?

Direct benefits

Direct benefits models predict that females mating with attractive mates gain by surviving longer or producing more eggs. Weak evidence is presented to suggest that females mating with popular males did indeed survive longer post-oviposition than

those mating with less successful males. However, females could only benefit if their greater post-oviposition survival translates directly into an increased probability of surviving to produce a second batch of eggs. It is known that in their natural environment females do survive multiple gonotrophic cycles (Dye *et al.* 1987). Nevertheless, in the laboratory females rarely survive to complete a second cycle (Chapter 2), so testing the significance of increased post-oviposition survival in the laboratory is difficult. As all other measures of survival and fecundity were unrelated to the numbers of matings obtained by mates, the biological significance of the post-oviposition result thus remains unclear.

Good-genes models

These models predict that choosy females gain good genes, coding for high viability for their offspring, by mating with attractive males. In this study no evidence was found to support this prediction. Sons and daughters sired by high ranking males did not survive longer, and daughters were not more fecund, than offspring sired by lower ranking males. One obvious potential problem in this study is that it is not known how well the laboratory environment reflects the field situation. In the laboratory, food is plentiful and potential intra-specific competition reduced, thus individuals may be relieved from the stresses normally imposed in their natural environment. Nevertheless, in this study, the laboratory environment was sufficiently harsh to reveal differences in the reproductive success of sons sired by different males. Moreover, the only weak trend found when exploring differences in offspring survival contradicted the prediction of good-genes models: fed daughters sired by preferred males tended to survive less well than those sired by unpreferred males.

A further potentially confounding variable in this sort of experiment is that females may invest differentially in their offspring if they are permitted to choose their preferred mate (Burley 1988, de Lope and Møller 1993). In their studies on birds Petrie (1994) and Norris (1993) controlled for potential differences in maternal investment using cross-fostering experiments. Petrie (1994) also found no differences

in the lipid or protein contents of eggs differing in paternity. In contrast, in field crickets, females mated to larger, preferred, males laid significantly more eggs than females mating with smaller less preferred males (Simmons 1987), confounding the interpretation of results which otherwise support a good-genes mechanism. In species such as sandflies where females lay a large number of eggs, it may be expected that differences in maternal investment would be reflected in the quantity rather than the quality of eggs produced. In the present study, females were consistently permitted a free choice of male and yet did not appear to differentially invest in offspring: the numbers of eggs laid was consistent across females.

Fisherian process

Fisherian models predict that females mating with attractive males should produce particularly attractive sons. In this study, sons sired by high-ranking males achieved more matings than sons sired by average and low ranking males, supporting the idea that a Fisherian mechanism promotes female choice in this species. To my knowledge this is the first study to provide evidence for heritability of male mating success in the absence of all other potential benefits. Whittier and Kaneshiro (1995) explicitly explored the relationship between father and son mating success in the Mediterranean fruit fly, and looked for potential direct benefits for females that mate with males that achieve high copulatory success. However, they found no evidence for a heritable component to male copulatory success and females mating with males that were generally preferred did not gain direct fitness benefits from their choice of mate through increased fertility. In addition, they did not attempt to measure offspring survival as a test of a possible good-genes mechanism.

A caveat in this experiment (and that of others: Norris 1993, Whittier and Kaneshiro 1995) is that I was unable to control for potential differences in the choosiness of females. In Fisherian models, choosy females carry genes for both a preference and the preferred male trait, which their sons will inherit (Fisher 1930). Attractive males may thus sire more attractive sons and a female may appear to benefit

indirectly from her choice of mate even though these differences may be generated entirely via maternal (rather than paternal) effects. To partially account for this problem, Petrie (1994) randomly assigned females to males of known attractiveness. In this study there are two specific reasons why this protocol was not followed. First, I wanted all females to experience choice, as it has been shown that differences may arise between those females permitted a choice and those females randomly allocated preferred males (Simmons 1987). Second, the attractiveness of males could not be judged *a priori* as no morphological correlate for male mating success within age classes had been isolated (but see Chapter 7), therefore it was necessary to judge male attractiveness directly from female choice. Despite this, it is hard to explain the inheritance of male attractiveness - either maternal or paternal - without invoking a Fisherian mechanism.

Conclusion

In conclusion, this experiment provides the first simultaneous test of the three main models for the evolution of female choice. Due to the potentially confounding effects discussed above, it is not possible to completely exclude good-genes or direct benefits models as additional mechanisms promoting female mate preference. In the absence of unequivocal support for the other hypotheses tested, however, the data suggest that a Fisherian mechanism is at least partially responsible for the evolution of female choice in this species. In the following chapter I explore several potential cues which might determine the observed variance in male mating success.

Chapter 7

Pheromones and mate choice

Summary

1. Pheromones are chemical signals transmitted between members of the same species. While traditionally they were thought to have evolved for species recognition, recent studies suggest that pheromone-mediated communication may play an important role in mate choice in some species.
2. In this chapter I investigate the possible role of pheromones in mate choice in the sandfly, *Lutzomyia longipalpis*. I also explore whether males or females displayed any behavioural traits that predict patterns of male mating success.
3. Pairs of virgin males were introduced into a small mating chamber and allowed to interact prior to the introduction of a single virgin female. All trials were recorded on video and later analysed for male and female behaviour. After each trial, the quantity of pheromone remaining in the glands of both males was determined by gas chromatography mass spectrometry and the pheromone glands and tergites were measured using a digitised measuring system.
4. Successful males had significantly more pheromone present in their glands after a trial than unsuccessful males. Mating success was also related to the proportion of time a male spent wing-fluttering, both in the presence and absence of females and males that had more pheromone also spent more time wing-fluttering. The amount of time invested in wing-fluttering was strongly correlated between males in a given pair, but other behavioural traits were not correlated, and a male's ability to win fights did not relate to his reproductive success. Females were equally likely to respond to both males. I found no relationship between the quantity of pheromone present in a male and the size of his gland.
5. To my knowledge, this is the first study to reveal a link between quantitative differences in the amount of pheromones produced by males and their reproductive success.
6. I conclude by discussing possible reasons for the variance observed in the amount of pheromone produced by individual males and suggest how female preferences for increased pheromone production and wing-fluttering may be maintained.

Introduction

Pheromones (from the Greek words *Pherein*, to transmit and *hormon*, to excite) are chemical signals transmitted between members of the same species. They tend to have specific chemical properties that are dependent on their use. For example air-borne pheromones need to be volatile; water-borne compounds require stability; and those chemicals designed to remain in the environment are necessarily persistent (Agosta 1992). Pheromones have long been recognised as important signals in animal communication (for reviews see Gosling 1990, Hölldobler and Wilson 1990, Macdonald *et al.* 1990, Agosta 1992, Alberts 1992). The traditional interpretation was that they evolved primarily as a mechanism for species recognition and mate attraction (for discussions see Thornhill and Alcock 1983, Birch *et al.* 1990). This view is, however, rapidly changing as their role in mate choice becomes increasingly apparent (Andersson 1994, Svensson 1996). In most species where pheromones have been specifically related to mate choice, males are the producers and pheromones either act directly to increase their attractiveness to females, or are correlated with some other preferred trait (Breed *et al.* 1980, Boake 1986, Moore and Breed 1986, Moore 1988, Houck and Regan 1990, Thornhill 1992, Lewis and Austad 1994, Shelly and Dewire 1994).

Pheromones have been shown to enhance a female's receptivity to males. In the cockroach, *Nauphoeta cinerea*, females are more likely to respond to the pheromones of dominant males (Moore and Breed 1986, Moore 1988). Similarly, in the plethodontid salamander, *Desmognathus ochrophaeus*, pheromones that are transferred directly from a male to a female during courtship increase female receptivity to male courtship and reduce the time to mating (Houck and Regan 1990). Pheromones have also been implicated as possible indicators of the physiological condition of male scorpionflies, *Panorpa japonica* (Thornhill 1992) and related to a male's probability of fertilising a female. For example, a study on flour beetles, *Tribolium castaneum*, revealed that male olfactory attractiveness was positively correlated with a male's subsequent fertilisation success (Lewis and Austad 1994), with attractive males achieving higher second male sperm

precedence. Finally, in the oriental fruit fly, *Bactrocera dorsalis*, a male's attractiveness to females and his wing-fluttering rate were both enhanced by providing him with chemicals that are utilised in the production of sex pheromones (Shelly and Dewire 1994). Control males, that were not provided with chemicals, had lower mating success and lower rates of wing-fluttering.

While all the above studies link pheromones to male mating success and there is increasing evidence that pheromones constitute an important cue used in mate choice, a gap remains in the literature. No study, to my knowledge, has so far attempted to quantify differences in the amounts of pheromones produced by successful and unsuccessful males, or explore whether the quantity of pheromone produced by males is correlated with any observed behavioural trait.

In the *Lutzomyia longipalpis* species complex, pheromones, that are dispersed from the abdominal glands of males, attract both sexes over long distances (Morton and Ward 1989, Ward *et al.* 1988, Elnaiem and Ward 1991, Kelly and Dye 1997; see Chapter 2). Sibling species can be identified by their pheromone type (Hamilton and Ward 1991) and it appears that pheromones are an effective reproductive isolating mechanism: although laboratory crosses between sibling species differing in pheromone type have produced viable, but less fertile hybrids (Ward *et al.* 1988, Ward and Morton 1991), hybrids have never been found in field captures (J.G.C. Hamilton pers. comm.). The mechanism of pheromone dispersal is as yet unconfirmed, but is suspected to involve wing-fluttering, as males frequently engage in this activity in isolation or in the presence of conspecifics (pers. obs.). Because only males produce pheromones and these are known to attract females, it was suspected that pheromones may also act as a stimulus in mate choice. Given a choice, females preferentially mate with middle-aged (4-6 d old) rather than very young (0-2d old) or very old (8-10d old) males (Chapter 5). Within middle-aged males, mating success is highly skewed and in part determined by female choice (Chapter 3).

This chapter presents the results of an experiment aimed at investigating the potential role of pheromones in female choice between *L. longipalpis* males of similar age. Specifically, I attempt to quantify differences in the amount of pheromones produced by successful (mated) and unsuccessful (unmated) males. The experiment was also designed to investigate whether males or females displayed behavioural traits that predicted patterns of male mating success.

Methods

All flies used in the study were virgin males and females released at less than 12hr post eclosion in small numbers (<30) into single-sex cages. They were maintained under standard laboratory conditions (Chapter 2) for 4-6d prior to use. As male mating success is skewed irrespective of female feeding status (Chapter 3) and I was not interested in assessing fecundity, females were not bloodfed prior to the experiment. Trials were run in the UK at 24^0C and 80% humidity, using infra-red light. Male mating behaviour is comparable under these conditions to mating behaviour observed under low light (pers. obs.).

To assess the role of pheromones on female choice and male mating success, a total of 32 trials were observed in a small mating chamber. This consisted of a rectangular perspex box (internal dimensions: length 3.9cm, width 4.1cm, height 1.3cm) with a convex base and removable perspex lid (which was thin enough to permit filming). A strip of filter paper was placed on the base of the chamber to provide a resting surface for the flies and small holes (0.6cm diameter) were drilled into both sides of the chamber, so that flies could be inserted using a glass tube. After introduction of flies, the holes were plugged with cottonwool to prevent escape.

For each trial, two new males were selected from the stock cage and introduced into the mating chamber. Males were left for approximately ten minutes prior to the introduction of a new female. The two males and single female were

then left until the female copulated with a male. Approximately ten seconds after copulation commenced (as copulations attempts are not usually prematurely terminated by a female depressing her abdomen after this time, pers obs.), all flies were anaesthetised using CO_2. The CO_2 was gently blown through a pipette that had been inserted into the chamber and aimed directly at the flies. Because flies became motionless immediately, this method allowed mated and unmated males to be identified and separated with 100% accuracy. Males were then killed by placing them into a -70^{0}C freezer. The mating chamber was cleaned with water and weak detergent to ensure that the presence of residual pheromones did not interfere with the outcome of successive trials. All trials were video recorded using an infra-red sensitive COHU camera linked to *The Observer* (Noldus Information Technology) timecode generator. The tapes were later analysed for instances of intra- and inter-sexual interactions (defined on Table 7.1) using *The Observer 3.1* software package.

Pheromone analysis

Pheromone analysis was carried out on a Hewlett Packard 5890 II+ gas chromatograph (GC) with an HP-5MS capillary column, 30mm x 0.25mm i.d., 0.25 mm film thickness, directly coupled to a Hewlett Packard 5972A benchtop mass spectrometer (MS), EI, 70eV, 165°C. The sample was introduced via a cool on-column injector (40°C). The GC was temperature programmed with an initial 2 min at 40°C, then an increase of 10°C min^{-1} to a final isothermal period at 250°C (10 min). After removal from the observation chamber males were placed separately in vials prepared from Pasteur pipettes, to which 15ml of hexane (pesticide grade, BDH, Poole, Dorset) was added. Each vial was then flame-sealed and left for a minimum of 24hr prior to analysis by GC-MS. Pheromone analysis was conducted blind to the outcome of the mating trials. Prior to injection into the GC, the solvent was reduced in volume to 1 ml. Males from the same trial were analysed consecutively to ensure maximum comparability between analyses. Pheromone peaks were unambiguously identified by mass spectrum and retention time. Peak areas were manually integrated using an integration utility within the GC-MS operating software. Pairs were discarded from analysis if the extraction

process or the injection were faulty for either male. Males were subsequently placed in 70% ethanol prior to measurement of the tergal gland and the corresponding tergite.

Table 7.1. Definitions of terms used to describe behaviour of two males and female.

Behaviour	Description of behaviour
Male wing-fluttering to male to female	Male fans wings while standing still or walking, either - at or near to a male, or - at a female
Fight	Contest between the males, usually preceded by a single male approaching the other and the two males clashing abdomens. Fights involved escalation to a contest where the males tumble over one another in an extended bout and usually lead to one male leaving the encounter site.
Winner	The male remaining at the site of a fight.
Loser	The male leaving the encounter site after the fight.
Female rejection	Female moves away from an approaching or wing-fluttering male.
Female wing-flutter	Female flutters her wings at an approaching male.

Gland measurements

In order to measure the width of the pheromone producing gland and tergite, the abdomen was dissected away from each male and mounted in Euparal (GBI Labs Ltd.) with the right side of the abdomen facing up. The right tergal gland was visible as a pale patch on the darkened tergite and was measured using a digitised computer system attached to a binocular microscope. Two measurements were taken, (i) the width of the gland, measured along the length of the tergite and (ii) the width of the chitin region of the fourth tergite. I measured all samples twice and used Interclass correlations to assess repeatability of the measurements (Zar 1984). Repeated measures of both gland and tergite width were highly correlated (gland width: $r_I = 0.95$, $F_{1,90} = 36.0$, $P < 0.001$; tergite width: $r_I = 0.88$, $F_{1,92} = 15.9$, $P < 0.01$).

The relationships between male and female behaviours (Table 7.1) and male mating success were compared using non-parametric pairwise comparisons as the data could not be transformed to normality. Correlations between behaviours were performed using Spearman rank correlation tests. After correction for multiple comparison the 95% significance level for behavioural comparisons is given by $P < 0.01$, and for behavioural correlations $P < 0.001$. The amount of pheromone extracted from mated and unmated males was compared using a pairwise non-parametric test. To assess whether the females commenced copulating more rapidly when the differences between the two males were greatest, female latency to mating was calculated as the time from introduction to the chamber to the onset of copulation and was compared with differences in wing-fluttering behaviour and pheromone content of the two males. The widths of the tergal glands and tergite of successful and unsuccessful males were compared using paired t-tests on untransformed data.

Results

Behavioural observations

Whether a male was successful was strongly related to the proportion of time that he spent wing-fluttering, both before and after the female was introduced (Table 7.2, Figures 7.1, 7.2). In contrast, a male's competitive ability, measured as the proportion of fights won, was unrelated to whether he mated with a female. Female behaviour was similar towards both males: with females rejecting and wing-fluttering at each male equally (Table 7.2).

Table 7.2. Comparisons of behaviours of successful and unsuccessful males. Uncorrected significance levels are given by: NS = not significant, ** = $P < 0.01$, *** = $P < 0.001$.

Behaviour	Median		Wilcoxon signed ranks test ($n = 32$)
	Successful	Unsuccessful	
Males present only			
Prop. of fights won	0.53	0.40	$Z = 1.87$ NS
Prop. of time spent wing-fluttering	0.28	0.19	$Z = 2.91$ **
Female introduced			
Prop. of fights won (✞)	0.33	0.50	$Z = 0.19$ NS
Prop. of time male spent wing-fluttering to other male	0.01	0.05	$Z = 2.16$ NS
Prop. of time male spent wing-fluttering to female	0.08	0.00	$Z = 3.88$ ***
No. of times female rejects male	0.00	0.00	$Z = 1.01$ NS
No. of times female wing-flutters to male	1.00	0.00	$Z = 1.67$ NS

(✞) $n = 11$, as analysis of the proportion of fights was restricted to those pairs of males that engaged in fights in the presence of females.

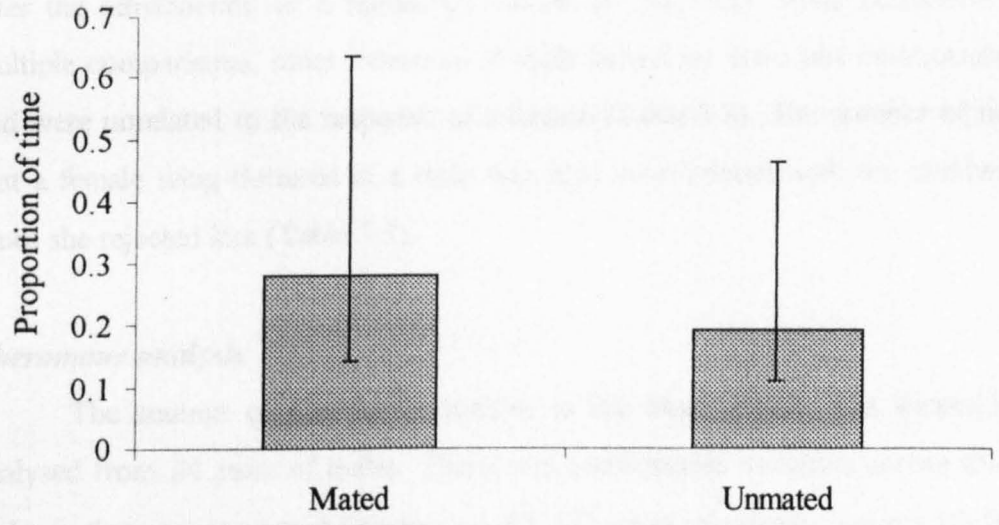


Figure 7.1. The proportion of time spent wing-fluttering by successful and unsuccessful males prior to the introduction of females. The columns represent the medians, and the error bars one interquartile range about the median.

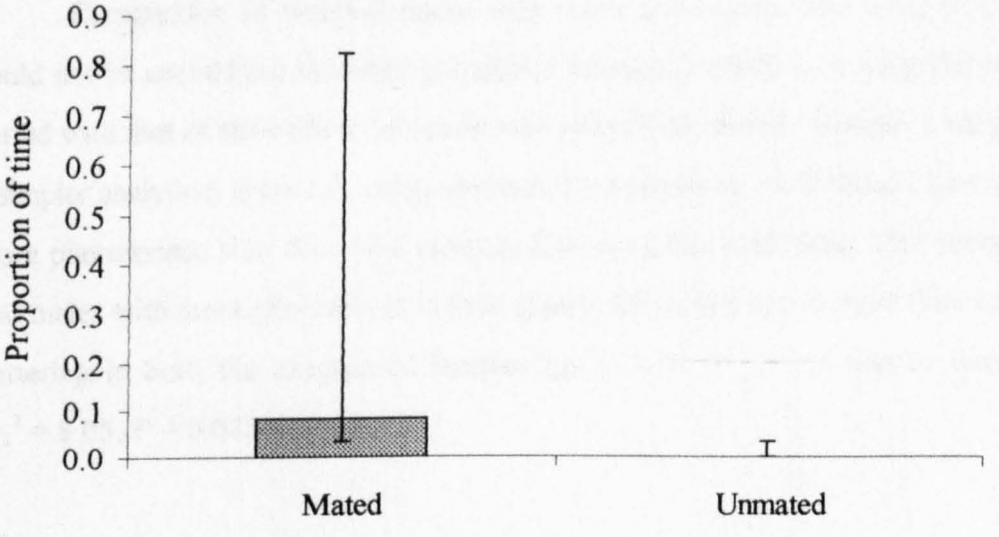


Figure 7.2. The proportion of time unsuccessful and successful males spent wing-fluttering to females. Notation as in Figure 7.1.

The proportion of time that a male spent wing-fluttering was positively correlated between males in a pair prior to (r_s = 0.67, n = 32, P <0.001), but not after the introduction of a female (r_s = 0.24, n = 32, NS). After correction for multiple comparisons, other measures of male behaviour were not intercorrelated and were unrelated to the response of a female (Table 7.3). The number of times that a female wing-fluttered at a male was also uncorrelated with the number of times she rejected him (Table 7.3).

Pheromone analysis

The amount of pheromone present in the tergal glands was successfully analysed from 24 pairs of males. There was considerable variation across the 48 males in the amount present (median = 1.97×10^{-6} ng/µl of solvent, range 3.10×10^{-4} - 8.86×10^{-6} ng/µl of solvent). Successful males had significantly greater amounts of pheromone than those males that were eventually rejected by females (median of successful males: 3.27×10^{-6} ng/µl of solvent; unsuccessful males: 2.13×10^{-6} ng/µl of solvent; Wilcoxon signed ranks test: Z = 2.34, n = 24, P < 0.02, Figure 7.3).

Comparison of whether males with more pheromone also wing-fluttered could not be carried out by direct correlation because a males own wing-fluttering varied with that of the male to which he was paired (see above). Instead, I adopted a simpler analytical approach, using contingency analysis to see if those males with more pheromones than their trial partners also wing-fluttered more. This revealed that males with more pheromones in their glands did indeed spend more time wing-fluttering in both the absence of females (χ_1^2 = 5.76, P < 0.05) and to females (χ_1^2 = 8.05, P < 0.025).

Table 7.3. Spearman rank correlation coefficients between male and female behaviour across all trials. Uncorrected significance given by: NS = not significant, * = $P < 0.05$.

Behaviour	*Males present*	*Females introduced*				
	Prop. time spent wing-fluttering to other male	Prop. of fights won	Prop. time spent wing-fluttering to other male	Prop. time spent wing-fluttering to female	No. of times female rejects male	No. times female wing-flutters to male
Males present						
Prop. fights won	0.25 NS	0.01 NS	0.23 NS	-0.05 NS	0.24 NS	0.22 NS
Prop. time spent wing-fluttering to other male		-0.9 NS	0.31 *	0.3 NS	0.14 NS	0.2 NS
Females introduced						
Prop. of fights won			0.1 NS	0.02 NS	0.22 NS	0.36 *
Prop. time spent wing-fluttering to other male				-0.08 NS	0.38 *	0.27 NS
Prop. time spent wing-fluttering to female					0.34 *	0.26 NS
No. of times female rejects male						0.25 NS

There was no evidence to suggest that females commenced copulating more rapidly when the differences between the two males in the amount of pheromones present in the glands or the proportion of time spent wing-fluttering were greatest (Spearman rank correlation tests: latency versus pheromones: r_s = -0.12; versus wing-fluttering in absence of females: $r_s = 0.44$; versus wing-fluttering directly to females: $r_s = 0.17$; for all $n = 24$, NS).

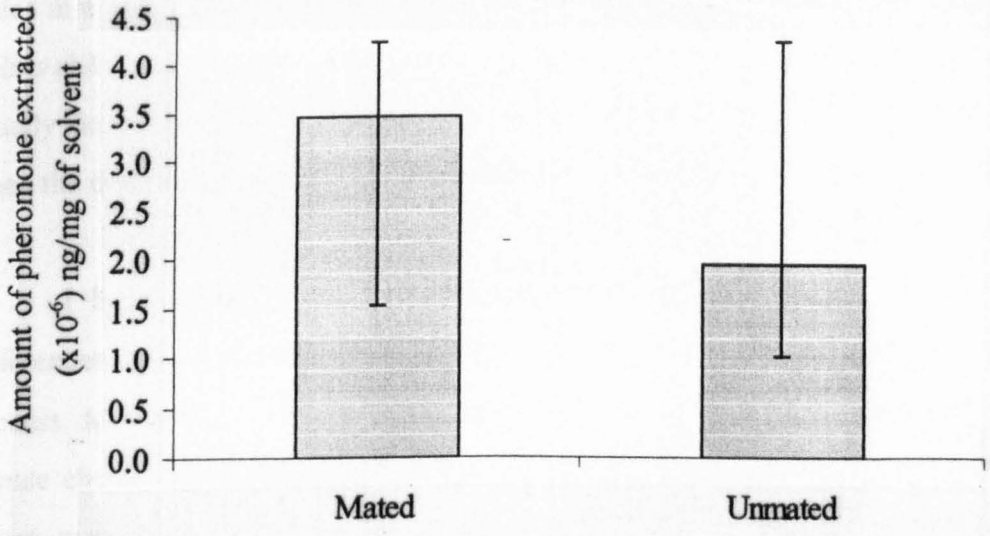


Figure 7.3. Amount of pheromone extracted from the glands of successful and unsuccessful males after choice trials. Notation as in Figure 7.1.

Tergal gland measurements

Male mating success was not related to either gland width (mean size ± s.e. successful: 0.14mm ± 0.003, unsuccessful 0.14mm ± 0.002; paired *t*-test: $t = 0.08$, n =20, NS) or tergite width (mean size ± s.e. successful: 0.20mm ± 0.003, unsuccessful 0.20mm ± 0.002; paired *t*-test: $t = 1.43$, $n = 20$, NS). There was also no relationship between the size of the gland and the amount of pheromone extracted from it (Spearman rank correlation test: r_s = -0.004, $n = 20$, NS).

Discussion

The most important findings of this study are that successful males had more pheromones present in their glands after a trial and spent a greater proportion of their time wing-fluttering during the trial, both in the presence and absence of females. The males in each pair with the most pheromone were also likely to spend more time wing-fluttering than their trial partners. Additionally the data indicated that the amount of time invested in wing-fluttering was strongly correlated between males in a given pair, but other behavioural traits were not intercorrelated, and a male's ability to win fights did not relate to his reproductive success. Females were equally likely to respond to both males and did not accept copulations more rapidly when the differences between the males were more marked.

I believe this is the first study to reveal a link between quantitative differences in the amount of pheromones produced by males and their reproductive success. Moreover, in this system, male success is at least partly determined by female choice: in previous experiments (Chapters 2 and 3), females were free to reject males, and in the above trials a male's mating success was entirely uncorrelated to his fighting ability. One alternative interpretation of the differences in pheromone content of males is that, as I measured the total amount of pheromone remaining in the glands rather than that released during a trial, successful males had more pheromone simply because they released less during the trial. However, as successful males also spent a greater proportion of their time wing-fluttering and hence probably dispersed more rather than less pheromone during the trial, this explanation seems unlikely. Instead these results suggest successful males both produce more pheromone and invest more time in dispersing it than do unsuccessful males.

The relative contribution made by wing-fluttering and pheromones in determining a male's reproductive success remains unknown. In *L. longipalpis*, pheromone production is not a pre-requisite of male mating success. Young males that do not possess pheromones (Boufana *et al.* 1986) are occasionally chosen by females in mate choice trials in preference to older, pheromone-producing, males

(Chapter 5). It is quite possible that females use both pheromone production and male display as cues in mate choice, with young males compensating for their lack of pheromones via increased wing-fluttering. If this were the case it would be expected that younger males would spend more time wing-fluttering than older males, at least when in their presence. Further investigation across males differing in age is needed to test this hypothesis.

An alternative explanation for the results in this chapter is that pheromones, while important in attracting females to aggregations of males, are then only correlated to those cues used in mate choice. Females have been found to use a hierarchy of cues in mate selection in other species (Wittenberger 1983, Gibson and Langen 1996). For example, in sage grouse, *Centrocercus urophasianus*, males attract females using vocal cues, but once at a site, females choose males using other cues such as display rate (Gibson 1996b). In the Caribbean fruit fly, *Anastrepha suspensa*, female attraction to a lek is pheromone-mediated, but once on a lek acoustic signals are thought to be responsible for mate choice (Sivinski *et al.* 1984). In *L. longipalpis*, both sexes produce song, although it's role in mate choice is as yet unquantified, thus it is conceivable that male pheromones may attract females to a particular lek, but other cues such as wing-fluttering or acoustic signalling may be invoked to select between available males.

Two other questions from these results. First, how did males differ so markedly in the amounts of pheromone they contained? There was no relationship between gland width and pheromone content. However, this may simply be due to gland depth being a more important determinant of the quantity of pheromone present than gland width. Alternatively, pheromone content may be entirely unrelated to gland size and depend instead on a male's nutritional status. Unlike the oriental fruitfly, *Bactrocera dorsalis* (McNeil and Delisle 1989, Shelly and Dewire 1994), the active compounds in the pheromones produced by *L. longipalpis* are not produced directly from ingested foods (J.G.C. Hamilton pers. comm.). Pheromone production might thus be related to general metabolic levels and hence

reflect a male's ability to forage for nectar. Elevated nutritional status may in turn also increase a male's ability to wing-flutter.

Second, if, as these results suggest, females do prefer males that produce more pheromone and wing-flutter more, how might this preference have evolved and be maintained? Current theoretical models suggest several possible mechanisms (reviewed in Chapter 6). If pheromone production and wing-fluttering are correlated with other traits, such as a male's ability to fertilise a female or survive, a female choosing to mate with a male that produces most pheromone and wing-flutters more may gain directly through increased probability of fertilisation or survival (Andersson 1994, Andersson and Iwasa 1996) or indirectly through the production of offspring with high survival (Zahavi 1975, Hamilton and Zuk 1982, Iwasa *et al.* 1991). Alternatively, a female may mate with a male that produces the most pheromone and wing-flutters more because he is simply more attractive and her sons will thus be more attractive to other females and achieve higher reproductive success (Fisher 1958, Pomiankowski *et al.* 1991). Finally, males may have exploited the physiological response of females to orientate to semiochemical and courtship cues, which were used initially in species recognition and host location (Arak and Enquist 1993, 1995, Johnstone 1995, Basolo 1995). In the previous chapter support was found for a Fisherian mechanism maintaining female mate preferences in this species: females mated to generally preferred males produced sons that achieved higher reproductive success than sons of less preferred males (Chapter 6). Moreover, females did not appear to gain any other fitness payoffs from their choice of mate (Chapter 6). As the fitness consequences of mate choice were not investigated here, it is not known whether pheromone production or a male's ability to wing-flutter are heritable, nor indeed whether they are correlated with male viability or confer any direct benefits to females.

Conclusion

In conclusion, the data indicate that male investment in pheromone production and wing-fluttering may be important cues used in mate choice in *L. longipalpis*. This is the first experiment to provide evidence that quantitative

differences in pheromones may be related to a male's reproductive success. Further work on the physiological costs and heritability of wing-fluttering and the production of pheromones is necessary to assess how far each contributes to a male's mating success and their relative importance in maintaining female mate preferences. It is likely that the variance in the amount of pheromone produced and the investment in wing-fluttering between males is greater in the field than in the relatively stable laboratory environment. Thus how far the results presented here relate to the field situation also remains to be tested.

Chapter 8

Conclusion

The aim of this thesis was to address fundamental questions in sexual selection and mating system theory using the sandfly *Lutzomyia longipalpis*. In Chapter 2, I provided baseline information on the natural history of the species. In the following chapter I investigate how well the mating system of *L. longipalpis* satisfied the criteria of a lekking species (Chapter 3). In Chapter 4, I explored how a lek-mating system may have evolved in this species by testing the predictions of several of the current models for lek evolution. In the final three data chapters (Chapters 5, 6 and 7), I looked for possible determinants of a male's reproductive success and then explored the consequences of a female's mate choice from males differing in age (Chapter 5) and from similar age males (Chapters 6 and 7). This concluding chapter summarises my main findings and suggests possible avenues for future research on sexual selection in *L. longipalpis*.

Mating system of Lutzomyia longipalpis

Laboratory experiments and field observations in Chapter 3 confirmed that *L. longipalpis* satisfies the necessary criteria of a lekking species (Bradbury 1981, 1985). Males clustered on or near hosts forming relatively stable aggregations which females visited for mating. While aggregations were near hosts, laboratory evidence suggested that the presence of a host, or a male's proximity to it had no effect on male reproductive success. Females were free to reject unwanted males and male mating success was typically highly skewed such that the majority of matings were distributed across a few males. Moreover, males were capable of fertilising several females without suffering from the effects of sperm depletion (Chapter 2). In contrast, females appear reluctant to mate more than once in a single gonotrophic cycle (Chapter 2), suggesting that sperm competition may not be an important determinant of patterns of sexual selection in this species. Moreover, this result suggests that permitting females single matings in the laboratory (the procedure usually followed in the experiments described in this thesis) is not unnatural for them, despite the fact that in the field they are exposed to several potential mates. In several lekking species the ability of a female to choose a mate freely has been questioned (Foster 1983, Trail 1985, Shelly 1987, Droney 1992, for reviews see Thornhill and Alcock 1983, Höglund and Alatalo

1995). Observations that female *L. longipalpis* can reject males both prior to and after genital contact (Chapter 3), and that a male's mating success was unrelated to his ability to win fights (Chapter 7) both suggest that female choice is unlikely to be constrained by male aggression. I was, however, unable to determine the relative contributions of male-male competition and female choice in determining a male's mating success (Chapter 3). For example, male-male interactions may be important in lek establishment: in the absence of females, male investment in wing-fluttering was positively correlated with their subsequent mating success (Chapter 6). However, such intra-sexual interactions clearly have only a partial effect in limiting a female's access to particular males, and female mate choice remains an important determinant of differential reproductive success in male *L. longipalpis*.

Evolution of lekking

In Chapter 4, I explored the evolution of the mating system of *L. longipalpis* by using field and laboratory data to test several of the predictions of current models for the evolution of lekking. The freedom to manipulate the availability of hosts enabled me to explore the relationship between the distribution of males and females in relation to resources. The data were best described by a female preference model combined with a hotspot-type mechanism: the distribution of males was influenced by the distribution of resources, while the number of females present in an aggregation correlated with lek size, but was less sensitive to the distribution of resources. Because no data were available on the reproductive success of males within leks, only tentative conclusions can be drawn with regards to male quality, and it is therefore difficult to further distinguish between each of the various models that are based on an underlying hotspot mechanism. A solution to this problem would be to mark and follow individuals in the field, but with small insects, and particularly species that are active in the dark, this is often not feasible. Alternatively, mating couples may be captured in *copula* (Markow and Ricker 1992, Partridge et al. 1987), however, this provides only a binary measure of mating success and thus may not be an accurate measure of a male's potential reproductive success on a lek.

Cues used in mate choice

In the final three chapters, I focused on the cues used in mate choice and the possible fitness consequences of female mating preferences. The evidence presented in Chapter 5 suggests that male mating success in *L. longipalpis* is age-dependent: given an opportunity to choose between males differing in age, females mated more frequently with middle-aged males than young or old males. Even within middle-aged males reproductive success was highly skewed (Chapters 3 and 6), but was unrelated to any measure of size taken (Chapter 6). The data in Chapter 7 indicated that male investment in pheromone production and wing-fluttering may be important determinants of a male's reproductive success: males with large quantities of pheromones present in their glands and that wing-fluttered most were more likely to obtain a mating (Chapter 7). This is the first study to quantify the relationship between a male's reproductive success and the amount of pheromone that he produces.

There are several limitations with these studies. First, because of the small quantities of pheromone involved I was only able to measure the amount of pheromone remaining in a male's gland at the end of the trial, rather than the amount emitted by a male during a trial. However, while present analytical techniques are not sensitive enough to measure pheromone emission of an individual *L. longipalpis* male, the fact that males that had the most pheromone remaining in their glands were also the males that wing-fluttered most during a trial (Chapter 7) suggests that these males also emitted most pheromone. Second, I did not attempt to investigate the relationship between pheromone production and age. Although pheromones are not produced immediately by adult males (Boufana *et al.* 1986), whether production varies further with age remains to be quantified. Third, in order to standardise the techniques as far as possible, in those chapters where possible correlates of male mating success were investigated (Chapters 5, 6 and 7) all males used were inexperienced virgin males. Thus, if females discriminate between mated and unmated males, on a lek, where males are potentially exposed to several hundred females in an evening (Kelly *et al.* 1996), a male's mating history may be an important determinant of his future reproductive success. Finally,

I have largely ignored the role of acoustic communication in mate choice. This was partially due to logistic and time constraints, but also because in *L. longipalpis* only males produce pheromones while both sexes produce song (Ward *et al.* 1988), thus pheromones seemed more likely to have a role in female choice. However, as short range communication could be determined solely by acoustic signalling (e.g. Sivinski *et al.* 1984), and cues may be interrelated (Gibson and Langen 1996), identification and simultaneous investigation of all potential cues are needed to understand the relative contribution of each in determining a male's mating success.

Consequences of female mate choice

To test the predictions of the various models for the maintenance of female preferences, I investigated the fitness consequences of female choice both across (Chapter 5) and within (Chapter 6) age groups. Investigation across age groups revealed that females randomly mated with young and middle-aged males gained a direct fitness payoff through increased probability of fertilisation: significantly more first instar larvae emerged from eggs sired by young and middle-aged males than from eggs sired by old males (Chapter 5). In addition, the fact that no differences were found in the fertility of males when females were allowed to choose between males, suggests that they may discriminate between males on the basis of fertility and so only choose to mate with older males when they are as fertile as young or middle-aged males present. Thus females apparently gain direct benefits by discriminating between males of different ages. However, it is important to note that in this experiment I did not examine whether these payoffs are supplemented by any indirect fitness benefits of choice between different age males.

In Chapter 6 I looked at female choice between males of the same age and simultaneously tested both direct and indirect benefits models of female choice. I explored the fitness consequences of a female's mate choice in terms of her own survival and fecundity and the survival and reproductive success of her offspring. The results supported the predictions of a Fisherian mechanism: females mated to males that were generally preferred by other females produced more attractive sons

who were in turn preferred in mate choice trials over sons sired by less attractive fathers. In the absence of support for the other mechanisms tested, the experiment provided evidence to suggest that a Fisherian mechanism is at least partially responsible for maintaining female preferences in *L. longipalpis*. Ideally similar data needs to be obtained over several generations and from more than one population to assess whether selection pressures are constant and more importantly whether heritability is repeatable (Wilkinson *et al.* 1990, Gilburn and Day 1994, Day *et al.* 1996, Gilburn *et al.* 1996).

A final point to address is that, while rearing conditions for adults and progeny were standardised as far as possible, the majority of the experiments in this thesis was carried out in the laboratory and there is an increasing call to test laboratory evidence with well designed field experiments. However, while it is true that the laboratory is at best a compromise for the field, the fact that potentially confounding variables can be more easily controlled for makes it an ideal environment to identify cues used in mate choice and discriminate between the different models of sexual selection. In addition many of the essential manipulations that I carried out (e.g. marking individuals, standardising the age of flies) and observations that I made (e.g. monitoring parent and offspring survival, or individual behaviour) would, at present, simply not have been feasible in the field. Similar experiments to those outlined in this thesis, imposing different levels of stress (for example, a reduced food supply, or changes in temperature) could provide a more realistic "field" comparison for the results obtained.

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